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(54) Title: ELONGATION FACTOR-2 KINASE (EF-2 KINASE) AND METHODS OF USE THEREFOR

(57) Abstract

A new superfamily of protein kinases has been discovered that centers around eukaryotic elongation factor-2 kinase (eEF-2 kinase). The proetin kinases of this new superfamily have the following characteristics: 1) sequence similarity to eEF-2 kinase; 2) no sequence similarity to the protein kinases of either the serine/threonine/tyrosine kinase or histidine kinase superfamily; and, 3) specifically phosphorylates α-helical regions of proteins as opposed to β-turns, as seen in all other protein kinases. Assays have been developed utilizing eEF-2 kinase and a phosphorylation target consisting of a novel α-helical 16-amino acid peptide sequence to facilitate high-throughput screening for compounds that can specifically inhibit this protein kinase that has been implicated tumor growth and other hyperproliferative disorders. Additionally, the disclosed invention includes assessing eEF-2 kinase levels for diagnostic purposes, and therapeutic formulations to inhibit eEG-2 kinase activity.

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ELONGATION FACTOR-2 KINASE (EF-2 KINASE) AND METHODS OF USE THEREFOR

FIELD OF THE INVENTION

This invention relates generally to the identification of a new superfamily of eukaryotic protein kinases and the use of one member of this superfamily, elongation factor-2 kinase (eEF-2 kinase), in assays to screen for specific inhibitors. Specifically, this invention provides an isolated nucleic acid encoding a heart protein kinase, a melanoma protein kinase and a ch4 protein kinase. Specific inhibitors of the eEF-2 kinase may be potent therapeutics for amelioration of malignant transformation. Additionally, sequences complementary to eEF-2 kinase may have therapeutic efficacy as antisense drugs or be used in gene therapy. Specifically, the invention relates to assays developed using the recombinant eEF-2 kinase to screen for inhibitors of phosphorylation of a peptide derived from the myosin heavy chain (MHC) protein.

BACKGROUND OF THE INVENTION

Protein phosphorylation plays a critical role in many cellular processes (Krebs (1994) *Trends Biochem. Sci.* 19:439; Hanks and Hunter, (1996) *FASEB J.* 9:576-596; Hardie and Hanks, (1995) *The Protein Kinase Facts Book* (Academic, London)). There are two well-characterized superfamilies of protein kinases, with most of the protein kinases belonging to the serine/threonine/tyrosine kinase superfamily (Hanks and Hunter, (1996); Hardie and Hanks, (1995)). The characterization of several hundred members of this superfamily revealed that they all share a similar structural organization of their catalytic domains which consist of twelve conserved subdomains (Hanks and Hunter, (1996); Hardie and Hanks, (1995)). The other superfamily is referred to as the histidine kinase superfamily and is involved in the prokaryotic two-component signal transduction system, acting as sensor components (Stock et al., (1989) *Microbiol. Rev.* 53:450-490; Parkinson and Kofoid, (1992) *Annu. Rev. Genet.* 26:71-112; Swanson, et al., (1994) *Trends Biochem. Sci.* 19:485-490). Recently, eukaryotic members of this superfamily have also been described (Chang et al., (1993) *Science* 263:539-544; Ota and Varshavsky, (1993) *Science* 262:566-569; Maeda et al.,

(1994) *Nature* 369:242-245). Mitochondrial protein kinases have also recently been described that show structural homology to the histidine kinases, but phosphorylate their substrates on serine (Popov et al., (1992) *J. Biol. Chem.* 267:13127-13130; Popov et al., (1993) *J. Biol. Chem.* 268:26602-22606). Finally, several new protein kinases have been reported that show a lack of homology with either of the kinase superfamilies (Maru and Witte, (1991) *Cell* 67:459-468; Beeler et al., (1994) *Mol. Cell. Biol.* 14:982-988; Dikstein et al., (1996) *Cell* 84:781-790; Futey et al., (1995) *J. Biol. Chem.* 270:523-529; Eichenger et al., (1996) *EMBO J.* 15:5547-5556). However, these protein kinases are viewed as an exception to the general rule as they have yet to be fully characterized.

The cloning and sequencing of the extensively characterized eukaryotic elongation factor-2 kinase (eEF-2 kinase) from a variety of eukaryotic organisms has now revealed the existence of a novel class of protein kinases (Ryazanov et al., (1997) *Proc. Natl. Acad. Sci., USA* 94:4884-4889). eEF-2 kinase, previously known as Ca^{2+}/calmodulin-dependent protein kinase III, is highly specific for phosphorylation of elongation factor-2 (eEF-2), an abundant cytoplasmic protein that catalyzes the movement of the ribosome along mRNA during translation in eukaryotic cells (reviewed in Ryazanov and Spirin, (1993) In *Translational Regulation of Gene Expression* (Plenum, New York) Vol. 2, pp. 433-455; Nairn and Palfrey, (1996) In *Translational Control* (CSHL Press, New York) pp. 295-318). All mammalian tissues, and various invertebrate organisms, exhibit eEF-2 kinase activity (Abdelmajid et al., (1993) *Int. J. Dev. Biol.* 37:279-290). eEF-2 kinase catalyzes the phosphorylation of eEF-2 at two highly conserved threonine residues located within a GTP-binding domain (Ryazanov and Spirin, (1993) In *Translational Regulation of Gene Expression* (Plenum, New York) Vol. 2, pp. 433-455; Nairn and Palfrey, (1996) In *Translational Control* (CSHL Press, New York) pp. 295-318). When eEF-2 is phosphorylated, it becomes inactive with respect to protein synthesis (Ryazanov et al., (1988) *Nature* 334:170-173). Since eEF-2 phosphorylation is dependent on Ca^{2+} and calmodulin, eEF-2 kinase plays a pivotal role in modulating the protein synthesis rate in response to changes in intracellular

calcium concentration. Phosphorylation of eEF-2 has also been linked to the regulation of cell cycle progression. For example, transient phosphorylation of eEF-2 occurs during the mitogenic stimulation of quiescent cells (Palfrey et al., (1987) *J. Biol. Chem.* 262:9785-9792) and during mitosis (Celis et al., (1990) *Proc. Natl. Acad. Sci., USA* 87:4231-4235). In addition, changes in the level of eEF-2 kinase activity is associated with a host of cellular processes such as cellular differentiation (End et al., (1982) *J. Biol. Chem.* 257:9223-9225; Koizumi et al., (1989) *FEBS Lett.* 253:55-58; Brady et al., (1990) *J. Neurochem.* 54:1034-1039), oogenesis (Severinov et al., (1990) *New Biol.* 2: 887-893), and malignant transformation (Bagaglio et al., (1993) *Cancer Res.* 53:2260-2264).

The sequence eEF-2 kinase appears to have no homology to either the Ca^{2+}/calmodulin-dependent protein kinases or to any members of the known protein kinase superfamilies (Ryazanov et al., (1997) *Proc. Natl. Acad. Sci., USA* 94:4884-4889). However, the recently described myosin heavy chain kinase A (MHCK A) from Dictyostelium (Futey et al., (1995) *J. Biol. Chem.* 270:523-529) shows a great deal of homology with eEF-2 kinase. These two kinases define a novel class of protein kinases that may represent a new superfamily.

Evidence for MHCK and eEF-2 kinase forming the core of a new superfamily is as follows. MHCK A from *Dictyostelium*, has a demonstrated role in the regulation of myosin assembly (Futey et al., (1995) *J. Biol. Chem.* 270:523-529; Côté et al., (1997) *J. Biol. Chem.* 272:6846-6849). eEF-2 kinase is a ubiquitous Ca^{2+}/calmodulin-dependant protein kinase involved in the regulation of protein synthesis by Ca^{2+} (Redpath et al., (1996) *J. Biol. Chem* 271:17547-17554; Ryazanov et al., (1997) *Proc. Natl. Acad. Sci., USA* 94:4884-4889). Both MHCK A and eEF-2 kinase display no homology to any of the known protein kinases, but are strikingly similar to each other; amino acid sequences of their catalytic domains are 40% identical. Another protein kinase homologous to MHCK A and eEF-2 kinase has recently been identified in *Dictyostelium* (Clancy et al., (1997) *J. Biol. Chem.* 272:11812-11815), and an

expressed sequence tag (EST) sequence, with a high degree of similarity to the catalytic domain common to both MHCK A and eEF-2 kinase, has been deposited in GenBank (clone FC-AN09/accession #C22986). An amino acid sequence alignment of the catalytic domains of these new protein kinases is shown in Figure 1A. These kinases have a catalytic domain of approximately 200 amino acids which can be subdivided into seven conserved subdomains. Subdomains V, VI, and VII have a predicted β-sheet structure and are presumably involved in ATP-binding, while subdomains I through IV may be involved in substrate binding and catalysis. These new protein kinases have no homology to the members of the eukaryotic serine/threonine/tyrosine protein kinase superfamily with the exception of the GXGXXG motif in subdomain VI which is present in many ATP-binding proteins. Thus, MHCK A, eEF-2 kinase, and related protein kinases may represent a new superfamily. Evolutionary analysis of these new kinases (Figure 1B) reveals that they can be subdivided into 2 families: the eEF-2 kinase family which includes eEF-2 kinases from different organisms, and the MHCK family which includes MHCK A, MHCK B and FC-AN09. These two families appear to have split more than a billion years ago.

An interesting question is why does nature employ these unusual kinases to phosphorylate eEF-2 and myosin heavy chains? Perhaps the answer is related to the secondary structure of the phosphorylation sites. As was originally reported by Small et al. (Small et al., (1977), *Biochim. Biophys. Res. Comm.* 79:341-346), phosphorylation sites are usually located at predicted β-turns. Subsequent studies, including X-ray crystallographic data, demonstrated that phosphoacceptor sites in substrates of conventional protein kinases are often located in turns or loops and usually have flexible extended conformation (Knighton et al., (1991) *Science* 253:414-420; Pinna and Ruzzene (1996) *Biochim. Biophys. Acta* 1314:191-225). In contrast to this, the existing evidence suggests that the peptides around phosphorylation sites for eEF-2 kinases and MHCK A have an α-helical conformation. The two major phosphorylation sites for MHCK A are located in a region which has a coiled-coil α-helical structure (Vaillancourt et al., (1988) *J. Biol. Chem.* 253:10082-10087). The major

phosphorylation site in eEF-2, threonine 56, is located within a sequence which is homologous among all translational elongation factors. In the crystal structure of the prokaryotic elongation factor EF-Tu, this sequence has an α-helical conformation (Polekhina *et al.*, (1996) *Structure* 4:1141-1151; Abel et al., (1996) *Structure* 4:1153-1159). These facts suggest that eEF-2 kinase and MHCK A differ from conventional protein kinases in that they phosphorylate amino acids located within α-helices.

Thus, in addition to the two well-characterized superfamily of eukaryotic protein kinases, which phosphorylate amino acids located in loops and turns, there appears to be a third superfamily of α-helix-directed kinases.

SUMMARY OF THE INVENTION

In accordance with the present invention, a new superfamily of protein kinases and corresponding methods for assaying their phosphorylation activity are disclosed. The protein kinases of this new superfamily have the following characteristics: 1) No significant sequence homology to protein kinases of either the serine/threonine/tyrosine kinase or histidine kinase super families; 2) moderate to high (≥40%) to eEF-2 kinases from any organism; and, 3) phosphorylates an amino acid within an α-helical domain.

The present invention also relates to a recombinant DNA molecule or cloned gene, or a degenerate variant thereof, which encodes eEF-2 kinase; preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding the eEF-2 kinase has a nucleotide sequence or is complementary to a DNA sequence shown in Figure 5 (SEQ ID NO: 1, 3, and 9).

The present invention also relates to an isolated nucleic acid molecule which encodes a heart protein kinase. The present invention also relates to an isolated nucleic acid molecule which encodes a melanoma protein kinase. The present invention also relates to an isolated nucleic acid molecule which encodes a ch4 protein kinase.

The human and murine DNA sequences of the eEF-2 kinase gene of the present invention or portions thereof, may be prepared as probes to screen for complementary sequences and genomic clones in the same or alternate species. The present invention extends to probes so prepared that may be provided for screening cDNA and genomic libraries for the eEF-2 kinase gene.

The present invention also includes eEF-2 kinase proteins having the activities noted herein, and that display the amino acid sequences set forth and described above and selected from SEQ ID NO: 2, 4, and 10.

The invention includes an assay system for screening of potential drugs effective at attenuating eEF-2 kinase activity of target mammalian cells by interrupting or potentiating the phosphorylation of eEF-2. It is a further object of the present invention to provide antibodies to the phosphorylated eEF-2 kinase target, and methods for their preparation, including recombinant means.

It is a further object of the present invention to provide a method for detecting eEF-2 kinase activity in mammals in which invasive, spontaneous, or idiopathic pathological states are suspected to be present.

It is a still further object of the present invention to provide a method for the treatment of mammals to control the amount or activity of eEF-2 kinase, so as to alter the adverse consequences of such presence or activity, or where beneficial, to enhance such activity.

It is a still further object of the present invention to provide a method for the treatment of mammals to control the amount or activity of eEF-2 kinase, so as to treat or avert the adverse consequences of invasive, spontaneous or idiopathic pathological states.

It is a still further object of the present invention to provide pharmaceutical compositions for use in therapeutic methods which comprise or are based upon a sequence complementary to that of the eEF-2 kinase mRNA, which would form the basis for an antisense therapeutic that can reduce expression, and thus activity, of eEF-2 kinase.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1B. (A) Sequence alignment of the catalytic domains of human eEF-2 kinase. *C. elegans* eEF-2 kinase, MHCK A, MHCK B and clone FC-ANO9. Identical amino acids (bold) and conserved hydrophobic amino acids (") are noted. (B). Phylogenetic tree of sequences shown in (A), with the addition of mouse and rat eEF-2 kinases. Tree was obtained using the J. Hein method with PAM250 residue weight table. The following accession numbers were used for the sequences: U93846-U93850, 1495779, 1170675, 1903458, C22986.

Figure 2. Expression of recombinant eEF-2 kinase *in vitro*. Plasmid DNA from clones *Cefk-1*, *Cefk-2*, as well as mouse and human eEF-2 kinase cDNA were used in the TNT wheat germ extract coupled transcription/translation system (Promega). [^{35}S]Methionine-labeled products were then analyzed by SDS/PAGE.

Figure 3. Activity of recombinant eEF-2 kinase *in vitro*. A large scale (0.5 ml) reaction using a mixture of *Cefk-1* and *Cefk-2* plasmids was run as in Figure 2, with the omission of labeled methionine. In the control experiment, the reaction was run with a plasmid containing a luciferase gene. (A) The reaction mixtures were separated by chromatography on a Mono Q column as described. (B) eEF-2 kinase activity in fractions was measured as the ability to phosphorylate purified rabbit eEF-2 in the

presence of [γ–^{32}P]ATP. Purified rabbit reticulocyte eEF-2 kinase was used in the (+) control experiments. (C) Ca^{2+}/calmodulin-dependency of recombinant *C. elegans* eEF-2 kinase. Mono Q fraction 25 was assayed in a standard eEF-2 kinase assay in the presence and absence of Ca^{2+} and calmodulin and 20 μM trifluoperazine (TFP) or *N*-(6 aminohexyl)-5-chloro-1-napthalene-sulfonamide (W7). (D) Ca^{2+}/calmodulin-dependency of recombinant human eEF-2 kinase. Human eEF-2 kinase cDNA was expressed in a coupled transcription/translation system as described above and eEF-2 kinase activity was assayed without further purification.

Figure 4. Northern blot analysis of tissue distribution of mouse eEF-2 kinase mRNA. Northern blots of mouse tissue containing 2 μg of polyadenylated RNA per lane were probed with the random-primed ^{32}P-labeled mouse eEF-2 kinase cDNA (31). The major transcript appeared at 3.1 kb and minor transcripts at 6.1 and 2.5 kb were also apparent (exposure time, 5 days). The same blots were stripped and rehybridized with a human eEF-2 cDNA (exposure time, 4 days).

Figure 5. Sequence alignment of *C. elegans*, mouse, human eEF-2 kinase, and the catalytic domain of *Dictyostelium discoideum* MHCK A. Identical amino acids are indicated by dark blue boxed regions and chemically conserved amino acids are indicated by light blue shaded regions. Amino acids in the human sequence that are identical to the mouse sequence are represented by dots. Amino acids underlined in black correspond to the six regions that match peptides obtained from the sequencing of purified rabbit reticulocyte eEF-2 kinase. The GXGXXG nucleotide-binding motif is underlined in red. The blue dashed line over residues 625-632 in *C. elegans* eEF-2 kinases designates the amino acids corresponding to exon 4, which is missing in *Cefk-2*.

Figure 6. Substrate specificity of eEF-2 kinase and MHCK A. Phosphorylation assays containing eEF-2 kinase (~50 ng) or MHCK A (0.2 μg) and either 0.5 μg rabbit reticulocyte eEF-2 or 0.1 μg *Dictyostelium* myosin were performed under standard conditions except that incubation time was extended to 10 min.

Figure 7. Schematic representation of the structure of mammalian and *C. elegans* eEF-2 kinases and MHCK A. The homologous regions are represented by dark shading. The regions of weak similarity are represented by light shading. The position of the GXGXXG motif is indicated by vertical arrows.

Figure 8. Assay for eEF-2 kinase activity. Recombinant eEF-2 kinase (2 μg) was incubated with increasing concentrations of a peptide phosphorylation target (RKKGESEKTKTKEFL) in a buffer consisting of 12.5 mM Hepes-KOH (pH 7.4), 2.5 mM magnesium acetate, 1.25 mM DTT, 25 μM $CaCl_2$, 0.5 μg calmodulin, 100 μM ATP, and 0.5 μCi [γ-^{33}P]ATP in a total volume of 50 μl. Samples were incubated at 30°C and aliquots were withdrawn at various time points, and the reaction was terminated by incubation in an ice water bath. The aliquots were then spotted onto phosphocellulose paper (2 cm x 2 cm) and washed (4 x 4 min) with 75 mM phosphoric acid. The papers were then rinsed with 100% ethanol, dried, and then counted in a scintillation counter.

Figure 9. Sequence alignment of *C. elegans*, mouse, human eEF-2 kinase, and the catalytic domain of *Dictyostelium discoideum* MHCK A, heart kinase, melanoma kinase and ch4 kinase. Identical amino acids are indicated by dark blue boxed regions and chemically conserved amino acids are indicated by light blue shaded regions.

Figure 10. pH-dependence of eEF-2 kinase activity: eEF-2 kinase activity was assayed by phosphorylation of the peptide substrate, MH-I at 0.2 pH unit intervals from pH 6.0-8.0.

Figure 11. Dependence of protein synthesis on intracellular pH: Protein synthesis was measured bu 3[H]-Leu-pulse incorporation in GH3 cells at various pH. Actual intracellular pH was determined by fluorimetric analysis.

Figure 12. Cytotoxicity assays of clones overexpressing eEF-2 kinase. MTT assays were done in quadruplicate and the results are presented as mean of values obtained.

Figure 13 Enzymatic activity of recombinant forms of human eEF-2 kinase expressed in *E. coli*. Autoradiograph of kinase assays with recombinant 6xHis-tagged eEF-2 kinase (lanes 1-4)- and GST-tagged-eEF-2 kinase (lanes 5-8). Reactions were done with and without eEF-2 (0.5 μg), and with and without calmodulin (0.5 μg). In autophosphorylation assays with 6xHis-eEF-2 kinase. 5 μg of eEF-2 kinase were used.

Figure 14 Enzymatic activity of eEF-2 kinase deletion mutants. Autoradiograph of eEF-2 kinase assay using total bacterial lysates of eEF-2 kinase deletion mutants. eEF-2 kinase assay was performed as described in text using 1 μg of total protein, and reactions were analyzed by 8% SDS-PAGE. Labels above lanes designate which amino acids are deleted in the mutant being assayed. Deletion of amino acids 51-335 causes loss of autophosphorylation activity, and a loss of eEF-2 kinase activity. Deletion of amino acids 521-725 also causes a loss of eEF-2 kinase activity.

Figure 15 Schematic representation of wild-type and mutant eEF-2 kinases. Hatched areas represent the putative catalytic domain. Black-shaded

areas represent regions conserved between various eEF-2 kinases. White-shaded areas represent nonconserved regions. Numbers at the top of the schematic represent the amino acids at the boundaries of each of these regions. eEF-2 kinase activity and autophosphorylation activity of each mutant are summarized on the right with (+) representing presence of activity and (-) representing absence of activity. n/d = not determined

Figures 16A-16B

(A) Phosphorylation of synthetic peptides by eEF-2 kinase. See text for experimental details. X-axis = time in minutes. Y-axis = ^{33}P incorporation into peptides in counts per minute. (B) Sequences of peptides corresponding to phosphorylation site in *Dictyostelium* myosin heavy chains (MHC, MH-1) and eEF-2. Phosphoacceptor threonines are designated by an asterisk. Helical wheel representation of both peptides is also shown. Phosphoacceptor threonines are circled.

DETAILED DESCRIPTION

Novel protein kinase inhibitors have the potential to form the basis for pharmaceutical compositions that can ameliorate malignant transformation. In order to find these inhibitors, libraries of chemical compounds are routinely screened using an automated protein kinase assay. The drawback to this approach is that most protein kinases have a very similar structure, thus making it difficult to specific inhibitors which act solely on a particular protein kinase. It was determined the primary structure of eEF-2 kinase, a ubiquitous enzyme which is involved in the regulation of protein synthesis and the cell cycle. Unexpectedly, it was found that eEF-2 kinase has a unique structure. It has no homology to any other mammalian protein kinase. This feature makes eEF-2 kinase an ideal target in the search for a specific protein kinase inhibitor. Since preliminary evidence suggests that eEF-2 kinase is upregulated in human cancers, including, but not limited to, breast cancer, identification of specific inhibitors of eEF-2 kinase can eventually lead to the development of novel anticancer drugs. In order be

able to perform a high throughput screen for an eEF-2 kinase inhibitor, it is first necessary to develop a simple assay which is amenable to automation. The existing assay involves incubation of partially purified eEF-2 kinase along with purified eEF-2 and [γ–^{32}P]ATP as substrates in the presence of increasing concentrations of candidate inhibitors. Results are then obtained by electrophoretic separation of the reaction mixtures, followed by autoradiography. Results are then quantified by either densitometry or scintillation counting of excised bands from the gel containing ^{32}P-eEF-2. Clearly, this assay, as it stands, is time-consuming, expensive, and not amenable to automation. Furthermore, it is difficult to purify large amounts of native eEF-2 required to perform multiple assays, and attempts to overexpress a recombinant form of eEF-2 were unsuccessful as its overexpression was toxic to host strains (personal communication from James Bodley, University of Minnesota, Minneapolis). Therefore, new methodologies for determining eEF-2 kinase activity were developed, which involves the use of a specific peptide substrate; easily and economically manufactured in large scale. These methods are relatively inexpensive, fast, and can be fully automated.

In the first attempt to use a peptide as an eEF-2 kinase substrate, peptides centered around the phosphorylation site of eEF-2 were generated. This strategy did not yield a peptide that was functional in phosphorylation assays. Surprisingly, it was found that a 16'mer peptide (RKKFGESEKTKTKEFL (SEQ ID NO: 20)), based on the phosphorylation site of *Dictyostelium discoideum* MHC, was an acceptable substrate for use with eEF-2 kinase in phosphorylation assays. It is interesting to note that while eEF-2 kinase can phosphorylate a peptide derived from MHC, it is not able to phosphorylate native MHC (Ryazanov et al., (1997) *Proc. Natl. Acad. Sci., USA* 94:4884-4889).

In accordance with the present invention, a new superfamily of protein kinases and corresponding methods for assaying their phosphorylation activity are disclosed. The protein kinases of this new superfamily have the following characteristics: 1) No

significant sequence homology to protein kinases of either the serine/threonine/tyrosine kinase or histidine kinase super families: 2) moderate to high (≥40%) to eEF-2 kinases from any organism; and, 3) phosphorylates an amino acid within an α-helical domain.

The present invention also relates to a recombinant DNA molecule or cloned gene, or a degenerate variant thereof, which encodes eEF-2 kinase; preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding the eEF-2 kinase has a nucleotide sequence or is complementary to a DNA sequence shown in Figure 5 (SEQ ID NO: 1, 3, and 9).

The human and murine DNA sequences of the eEF-2 kinase gene of the present invention or portions thereof, may be prepared as probes to screen for complementary sequences and genomic clones in the same or alternate species. The present invention extends to probes so prepared that may be provided for screening cDNA and genomic libraries for the eEF-2 kinase gene. For example, the probes may be prepared with a variety of known vectors, such as the phage λ vector. The present invention also includes the preparation of plasmids including such vectors, and the use of the DNA sequences to construct vectors expressing antisense RNA or ribozymes which would attack the mRNAs of any or all of the DNA sequences set forth in FIGURES 5 (SEQ ID NO: 1, 3, and 9). Correspondingly, the preparation of antisense RNA and ribozymes are included herein.

The present invention also includes eEF-2 kinase proteins having the activities noted herein, and that display the amino acid sequences set forth and described above and selected from SEQ ID NO: 2, 4, and 10.

This invention provides an isolated nucleic acid which encodes a heart protein kinase, including analogs, fragments, variants. and mutants, thereof. In one embodiment the nucleic acid has a nucleotide sequence having at least 90% similarity with the nucleic acid coding sequence as shown in Figure 9.In one embodiment the nucleic acid has a

nucleotide sequence having at least 85% similarity with the nucleic acid coding sequence as shown in Figure 9.In one embodiment the nucleic acid has a nucleotide sequence having at least 80% similarity with the nucleic acid coding sequence as shown in Figure 9. In another embodiment the nucleic acid has the following sequence:

CACTTGACTGCAGGGATAAAGAAGAAAATTCTATCCAGGGTCGC
AGCCCTGAGACTGAGGCTGGAGGAAAAGGAAAATTCGAGGAAG
AACTCCATCGTGAAGAAGACACCTAAGTTTGAAAGGTCCTTATC
CCGCACTGATGAGAAAAGAGACCCCAAAAGGGCCCCTTGCAAA
GCTGAAGGGAAAGCTCCAGTATTGCTGAAGAGGATCCAGGCCGA
GATGGCTCCCGAGCACTCCGGAAATATAAAGTTGAGCTGCCAGTTT TCAGA
AATCCATGAAGACTCTACCGTCTGCTGGACAAAAGATTCCAAGTCGATAG
CCCAGGCCAAGAAAAGCGCAGGGGACAACTCCAGTGTTTCCTTGGCCATC
GTCCAAGCTGGTCAGAAGGACCAGGGCCTGTATTACTGCTGCCTCAAGAA
CAGTTATGGAAAAGTCACTGCTGAGTTTAACCTCACAGCTGAAGTTCTCAA
ACAGCTTTCAAGTCACCAGAATACTAGAGGATGTGAAGAGATTGAATTCA
GCCAGCTCATCTTCAAAGAAGATGTTTTCAATGACAGCTACTTCGGGGAC
CACCTACGTGGCCAGATCTCCACGGAGGAGCTTCACTTTGGCGAAGGGGT
GCACCGCAAAGCTTTCCGGAGCAAGGTGATGCAGGGCCTCATGCCGGTCT
TCCAGCCCGGCCACGCATGCGTACTCAAGGTGCACAATGCCGTCGCCCAT
GGGACCAGAAACAATGACGAACTTGTGCAGAGGAACTACAAACTGGCTG
CCCAGGAATGCTACGTGCAGAATACTGCCAGATACTACGCCAAGATCTAC
GCCGCTGAAGCACAGCCTCTGGAAGGCTTCGGAGAGGTGCCGGAGATCAT
TCCTATTTTCCTTATCCATCGGCCCGAGAACAACATCCCATATGCCACAGT
GGAAGAAGAGCTGATTGGAGAATTCGTGAAGTATTCCATCCGGGACGGGA
AGGAAATCAACTTCCTCAGACGAGATTCAGAGGCTGGCCAGAAATGTTGC
ACCTTCCAGCACTGGGTATACCAGAAAACAAGTGGCTGTCTCCTGGTSAC
GGACATGCAGGGTGTGGGAATGAAGTTAACTGACGTTGGCATAGCAACAC
TAGCTAGAGGGTACAAAGGATTTAAGGGCAACTGTTCCATGACCTTCATT
GATCAGTTCAGAGCGCTGCATCAGTGTAACAAGTACTGTAAAATGCTGGG
GCTGAAATCCCTTCAAAACAACAGCCAGAAGCCCAGGAAGCCCATCGTCG

GGAAAGGCAGGGTTCCGACAAACGCCACGCAGGTGAAGACGCCTGAGTC
TGAGACGCCGCCCGCAGAAAGAAAAACCTAGCCTCCCTCCTCCCTTCATC
ACCAGTGACCACCAAGCCAGCATCGCGCAGGCTTGCGCGTGGACATCTGC
AAGCACACAAGGGACACGAGCCTGCAGCCTGCAGCCGAGTGCCAGTCCTC
TCAGCTCCTATCACTGGCTGTCTGCTGAAATGACAATGGCATGGCTCTTCC
AGACTAGCCTTGTAGAGAGACTTAGCAGTTCTGTTGATGCTCTCAAAGGC
AGCCCACTGTTTGTGTACACAGCTAGCCTTTCTACACACACCCTCCCCTCC
CACCGCATCGTCTATCTATCTGTGTGTCGCGCGTGGTTTGTTGACAAGAGT
TCCCCCGCTGCCTTGGCGACTGGCCACTGTCAAAATCCTTCCCACCTCGAC
CCCCTCACCTCAGGATGTTCCTGCAGTCATGAATGTCAAGTTGTTGTTATC
AGTGTCACCGACGCTATTGTTGCTGGAGGCGGCTTCCCAGATGCGAGCCC
ATTTCCCGCCACTACCCACGCAGCCTGGCACAGTGTTCTGTTTCATTAAAT
TCATATTTAAGCAAAAAAAAAAAAAAAAAA

This invention provides a heart protein kinase which has the amino acid sequence as follows:

HLTAGIKKKILSRVAALRLRLEEKENSRKNSIVKKTPKFERSLSRTDEKRDPKR
APCKAEGKAPVLLKRIQAEMAPEHSGNIKLSCQFSEIHEDSTVCWTKDSKSIA
QAKKSAGDNSSVSLAIVQAGQKDQGLYYCCLKNSYGKVTAEFNLTAEVLKQ
LSSHQNTRGCEEIEFSQLIFKEDVFNDSYFGDHLRGQISTEELHFGEGVHRKAF
RSKVMQGLMPVFQPGHACVLKVHNAVAHGTRNNDELVQRNYKLAAQECY
VQNTARYYAKIYAAEAQPLEGFGEVPEIIPIFLIHRPENNIPYATVEEELIGEFV
KYSIRDGKEINFLRRDSEAGQKCCTFQHWVYQKTSGCLLVTDMQGVGMKLT
DVGIATLARGYKGFKGNCSMTFIDQFRALHQCNKYCKMLGLKSLQNNSQKP
RKPIVGKGRVPTNATQVKTPESETPPAERKT

This invention provides an isolated nucleic acid which encodes a melanoma protein kinase, including analogs, fragments, variants, and mutants, thereof. In one embodiment the nucleic acid has a nucleotide sequence having at least 90% similarity with the nucleic acid coding sequence as shown in Figure 9.In one embodiment the nucleic acid has a

nucleotide sequence having at least 85% similarity with the nucleic acid coding sequence as shown in Figure 9.In one embodiment the nucleic acid has a nucleotide sequence having at least 80% similarity with the nucleic acid coding sequence as shown in Figure 9. On another embodiment the melnoma kinase has the following sequence:

GGCACGAGCTCGTCCACATATTACTATTATTCAGCTGTGGAAAGAAATAAC
CTGATGAGGTTGTCACAGAGTATTCCCTTCGTTCCTGTACCTCCACGAGGCG
GCCTGTCACAGTGTCCCGTCTGGAGGAGAGTTCTCCCAGTATACTGAATA
ACAGCATGTCTTCATGGTCTCAGCTAGGCCTCTGTGCCAAAATTGAGTTTT
TAAGTAAAGAGGAAATGGGAGGCGGTTTACGAAGAGCAGTCAAAGTGCT
GTGTACCTGGTCAGAGCACGATATCCTGAAGTCAGGGCATCTCTATATCAT
TAAGTCATTTCTTCCTGAGGTGATAAACACATGGTCAAGCATTTATAAAGA
AGATACGGTTCTACATCTCTGTCTCAGAGAAATACAACAACAGAGAGCAG
CACAAAAGCTCACATTTGCCTTTAATCAGATGAAACCCAAATCCATACCA
TATTCTCCAAGGTTCCTTGAAGTTTTCCTGTTGTACTGCCATTCAGCAGGG
CAGTGGTTCGCTGTAGAAGAGTGCATGACTGGTGAATTTAGAAAATACAA
CAACAATAATGGTGATGAAATCATTCCTACAAATACTCTAGAAGAGATCA
TGCTAGCCTTTAGCCACTGGACCTATGAATATACCAGAGGGGAGTTACTG
GTACTTGACTTACAAGGAGTGGGAGAAAACTTGACTGACCCATCTGTAAT
AAAAGCTGAAGAAAAAAGATCCTGTGACATGGTTTTTGGCCCTGCCAATC
TAGGAGAAGATGCAATAAAAAACTTCAGAGCCAAACATCACTGTAATTCT
TGCTGTCGAAAGCTTAAACTTCCAGATTTGAAGAGGAATGACTACACGCC
TGATAAAATTATATTTCCTCAGGATGAGTCATCAGATTTGAATCTTCAATC
TGGAAATTCCACCAAAGAATCAGAAGCAACAAATTCTGTTCGTCTGATGTTA

This invention provides a melanoma kinase protein which has the sequence as follows:
GTSSSTYYYYSAVERNNLMRLSQSIPFVPVPPRGEPVTVSRLEESSPSILNNSM
SSWSQLGLCAKIEFLSKEEMGGGLRRAVKVLCTWSEHDILKSGHLYIIKSFLPE
VINTWSSIYKEDTVLHLCLREIQQQRAAQKLTFAFNQMKPKSIPYSPRFLEVFL
LYCHSAGQWFAVEECMTGEFRKYNNNNGDEIIPTNTLEEIMLAFSHWTYEYT
RGELLVLDLQGVGENLTDPSVIKAEEKRSCDMVFGPANLGEDAIKNFRAKHHC

NSCCRKLKLPDLKRNDYTPDKIIFPQDESSDLNLQSGNSTKESEATNSVRLML

This invention provides an isolated nucleic acid which encodes a ch4 protein kinase, including analogs, fragments, variants, and mutants, thereof. In one embodiment the nucleic acid has a nucleotide sequence having at least 90% similarity with the nucleic acid coding sequence as shown in Figure 9.In one embodiment the nucleic acid has a nucleotide sequence having at least 85% similarity with the nucleic acid coding sequence as shown in Figure 9.In one embodiment the nucleic acid has a nucleotide sequence having at least 80% similarity with the nucleic acid coding sequence as shown in Figure 9. In another embodiment the ch 4 kinase has a nucleic acid which encodes the following amino acid sequence:

MCRKRTRARTSAAEASLRASILARDCAAAAAIVFLVDRFLYGLDVSGKLLQV
KGLHKLQPATPIAPQVVIRQARISVNSGKLLKAEYILSSLISNNGATGTWLYRN
ESDKVLVQSVCIQIRGQILQKLGMWYEAAELIWASIVGYLALPQPDKKGLSTS
LGILADIFVSMSKNDYEKFKNNPQINLLSLLKEFDHHLLSAAEACKLAAAFSA
YTPLFVLTAVNIRGTCLLSYSSSNDCPPELKNLHLCEAKEAFEIGLLTKRDDEP
VTGKQELHSFVKAAFGLTTVHRRLHGETGTVHAASQLCKEAMGKLYNFSTSSR
SQDREALSQEVMSVIAQVKEHLQVQSFSNVDDRSYVPESFECRLDKLILHGQGDF
QKILDTYSQHHTSVCEVFESDCGNNKNEQKDAKTGVCITALKTEIKNIDTVST
TQEKPHCQRDTGISSSLMGKNVQRELRRGGRRNWTHSDAFRVSLDQDVETET
EPSDYSNGEGAVFNKSLSGSQTSSAWSNLSGFSSSASWEEVNYHVDDRSARK
EPGKEHLVDTQCSTALSEELENDREGRAMHSLHSQLHDLSLQEPNNDNLEPS
QNQPQQQMPLTPFSPHNTPGIFLAPGAGLLEGAPEGIQEVRNMGPRNTSAHSR
PSYRSASWSSDSGRPKNMGTHPSVQKEEAFEIIVEFPETNCDVKDRQGKEQGE
EISERGAGPTFKASPSWVDPEGETAESTEDAPLDFHRVLHNSLGNISMLPCSSF
TPNWPVQNPDSRKSGGPVAEQGIDPDASTVDEEGQLLDSMDVPCTNGHGSH
RLCILRQPPGQRAETPNSSVSGNILFPVLSEDCTTTEEGNQPGNMLNCSQNSSS
SSVWWLKSPAFSSGSSEGDSPWSYLNSSGSSWVSLPGKMRKEILEARTLQPD
DFEKLLAGVRHDWLFQRLENTGVFKPSQLHRAHSLLLKYSKKSELWTAQETI
VYLGDYLTVKKKGRQRNAFWVHHLHQEEILGRYVGKDYKEQKGLWHHFTD

VERQMTAQHYVTEFNKRLYEQNIPTQIFYIPSTILLILEDKTIKGCISVEPYILGE
FVKLSNNTKVVKTEYKATEYGLAYGHFSYEFSNHRDVVVDLQGWVTGNGK
GLIYLTDPQIHSVDQKVFTTNFGKRGIFYFFNNQHVECNEICHRLSLTRPSMEKPX

In a further embodiment of the invention, the full DNA sequence of the recombinant DNA molecule or cloned gene so determined may be operatively linked to an expression control sequence which may be introduced into an appropriate host. The invention accordingly extends to unicellular hosts transformed with the cloned gene or recombinant DNA molecule comprising a DNA sequence encoding eEF-2 kinase, and more particularly, the complete DNA sequence determined from the sequences set forth above and in SEQ ID NO: 1, 3, and 9.

According to other preferred features of certain preferred embodiments of the present invention, a recombinant expression system is provided to produce biologically active animal or human eEF-2 kinase.

The present invention naturally contemplates several means for preparation of eEF-2 kinase, including as illustrated herein known recombinant techniques, and the invention is accordingly intended to cover such synthetic preparations within its scope. The isolation of the cDNA and amino acid sequences disclosed herein facilitates the production of eEF-2 kinase by such recombinant techniques, and accordingly, the invention extends to expression vectors prepared from the disclosed DNA sequences for expression in host systems by recombinant DNA techniques, and to the resulting transformed hosts.

The invention includes an assay system for screening of potential drugs effective at attenuating eEF-2 kinase activity of target mammalian cells by interrupting or potentiating the phosphorylation of eEF-2. In one instance, the test drug could be administered to a cellular sample along with ATP carrying a detectable label on its γ-phosphate that gets transferred to eEF-2, or a peptide substrate, by eEF-2 kinase.

Quantification of the labeled eEF-2 or peptide substrate is diagnostic of the candidate drug's efficacy. A further embodiment would provide for the assay to be performed using a purely *in vitro* system comprised of eEF-2 kinase , ATP or labeled ATP, eEF-2 or peptide analog of a portion of eEF-2 or MHC. appropriate buffer, and detection reagents and/or instrumentation to detect and quantify the extent of eEF-2 kinase-directed phosphorylation activity..

The assay system could more importantly be adapted to identify drugs or other entities that are capable of binding to the eEF-2 kinase and/or its cognate phosphorylation target (e.g. eEF-2), either in the cytoplasm or in the nucleus, thereby inhibiting or potentiating eEF-2 kinase activity and its resultant phenotypic outcome. Such an assay would be useful in the development of drugs that would be specific against particular cellular activity, or that would potentiate such activity, in time or in level of activity. For example, such drugs might be used to treat various carcinomas or other hyperproliferitive pathologies.

The present invention likewise extends to antibodies against specifically phosphorylated eEF-2 kinase targets (e.g. eEF-2 or peptide). including naturally raised and recombinantly prepared antibodies. These antibodies and there labeled counterparts are included within the scope of the present invention for their particular ability in detecting eEF-2 kinase activity *via* detection of the phosphorylated product by ELISA or any other immunoassay known to the skilled artisan.

In the instance where a radioactive label, such as the isotopes ^{3}H, ^{14}C, ^{32}P, ^{33}P. ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{90}Y, ^{125}I, ^{131}I, and ^{186}Re are used, known currently available counting procedures may be utilized. In the instance where the label is an enzyme, detection may be accomplished by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques known in the art.

In a further embodiment, the present invention contemplates antagonists of the activity of eEF-2 kinase. In particular, an agent or molecule that inhibits phosphorylation of eEF-2. In a specific embodiment, the antagonist can be a peptide comprising sequences, or sequence variants adjacent to, and including, the phosphorylation site in either eEF-2 or MHC. It is anticipated that these peptides would be competitive inhibitors of eEF-2 kinase's cognate target.

In still a further embodiment, the invention contemplates antisense drugs such that sequences complementary to the eEF-2 kinase mRNA inhibit production of functional eEF-2 kinase. In a specific embodiment, the antisense drug may be a complementary oligonucleotide (DNA, RNA, or hybrid thereof), which may or may not be modified so as to have the following characteristics: 1) enhanced hybridization kinetics; 2) tighter binding to complementary sequence than its unmodified counterpart; and/or, 3) resistance to nucleases. In another specific embodiment, the antisense drug may be a complementary oligonucleotide (DNA, RNA, or hybrid thereof), that has the ability to cleave its target sequence either by ribozyme, or ribozyme-like, activity, or by nuclease activity imparted on the antisense drug by physical attachment to anyone of a number of nucleases.

More specifically, the therapeutic method generally referred to herein could include the method for the treatment of various pathologies or other cellular dysfunctions and derangements by the administration of pharmaceutical compositions that may comprise effective inhibitors of eEF-2 kinase activity, or other equally effective drugs developed for instance by a drug screening assay prepared and used in accordance with a further aspect of the present invention.

Accordingly, it is a principal object of the present invention to provide a method and an associated assay system for screening potential inhibitors of eEF-2 kinase activity.

It is a further object of the present invention to provide antibodies to the phosphorylated eEF-2 kinase target, and methods for their preparation, including recombinant means.

It is a further object of the present invention to provide a method for detecting eEF-2 kinase activity in mammals in which invasive, spontaneous, or idiopathic pathological states are suspected to be present.

It is a still further object of the present invention to provide a method for the treatment of mammals to control the amount or activity of eEF-2 kinase, so as to alter the adverse consequences of such presence or activity, or where beneficial, to enhance such activity.

It is a still further object of the present invention to provide a method for the treatment of mammals to control the amount or activity of eEF-2 kinase, so as to treat or avert the adverse consequences of invasive, spontaneous or idiopathic pathological states.

It is a still further object of the present invention to provide pharmaceutical compositions for use in therapeutic methods which comprise or are based upon a sequence complementary to that of the eEF-2 kinase mRNA, which would form the basis for an antisense therapeutic that can reduce expression, and thus activity, of eEF-2 kinase.

It is yet another object of the invention to provide pharmaceutical compositions for use in therapeutic methods which comprise or are based upon peptide analogs of eEF-2 phosphorylation target amino acid sequences. It is anticipated that certain peptide analogs may act as efficacious competitive inhibitors of eEF-2 phosphorylation.

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook et al, "Molecular Cloning: A Laboratory Manual" (1989); "Current Protocols in Molecular Biology" Volumes I-III [Ausubel, R. M., ed. (1994)]; "Cell Biology: A Laboratory Handbook" Volumes I-III [J. E. Celis, ed. (1994))]; "Current Protocols in Immunology" Volumes I-

III [Coligan, J. E., ed. (1994)]; "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. (1985)]; "Transcription And Translation" [B.D. Hames & S.J. Higgins, eds. (1984)]; "Animal Cell Culture" [R.I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press. (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984).

Therefore. if appearing herein, the following terms shall have the definitions set out below.

The terms "elongation factor-2 kinase", "eEF-2 kinase ", "EF-2 kinase". "Cefk", and any variants not specifically listed, may be used herein interchangeably, and as used throughout the present application and claims refer to proteinaceous material including single or multiple proteins, and extends to those proteins having the amino acid sequence data described herein and presented in FIGURES 1 and 5 (SEQ ID NO: 2, 4, 6, 8, 10, 12, abd 14), and the profile of activities set forth herein and in the Claims. Accordingly, proteins displaying substantially equivalent or altered activity are likewise contemplated. These modifications may be deliberate. for example, such as modifications obtained through site-directed mutagenesis, or may be accidental, such as those obtained through mutations in hosts that are producers of the complex or its named subunits. Also, the terms elongation factor-2 kinase", "eEF-2 kinase". "EF-2 kinase". and "Cefk" are intended to include within their scope proteins specifically recited herein as well as all substantially homologous analogs and allelic variations.

The amino acid residues described herein are preferred to be in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired fractional property of immunoglobulin-binding is retained by the polypeptide. NH_2 refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a polypeptide. In keeping with standard polypeptide nomenclature, *J. Biol. Chem.*,

243:3552-59 (1969). abbreviations for amino acid residues are shown in the following Table of Correspondence:

TABLE OF CORRESPONDENCE

SYMBOL		AMINO ACID
1-Letter	3-Letter	
Y	Tyr	tyrosine
G	Gly	glycine
F	Phe	phenylalanine
M	Met	methionine
A	Ala	alanine
S	Ser	serine
I	Ile	isoleucine
L	Leu	leucine
T	Thr	threonine
V	Val	valine
P	Pro	proline
K	Lys	lysine
H	His	histidine
Q	Gln	glutamine
E	Glu	glutamic acid
W	Trp	tryptophan
R	Arg	arginine
D	Asp	aspartic acid
N	Asn	asparagine
C	Cys	cysteine

It should be noted that all amino-acid residue sequences are represented herein by formulae whose left and right orientation is in the conventional direction of amino-terminus to carboxy-terminus. Furthermore, it should be noted that a dash at the

beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino-acid residues. The above Table is presented to correlate the three-letter and one-letter notations which may appear alternately herein.

A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*; *i.e.*, capable of replication under its own control.

A "vector" is a replicon, such as plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single stranded form, or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA).

An "origin of replication" refers to those DNA sequences that participate in DNA synthesis.

A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g.,

mammalian) DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

An "expression control sequence" is a DNA sequence that controls and regulates the transcription and translation of another DNA sequence. A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence.

A "signal sequence" can be included before the coding sequence. This sequence encodes a signal peptide, N-terminal to the polypeptide, that communicates to the host cell to direct the polypeptide to the cell surface or secrete the polypeptide into the media, and this signal peptide is clipped off by the host cell before the protein leaves the cell. Signal

sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

The term "oligonucleotide," as used herein in referring to the probe of the present invention, is defined as a molecule comprised of two or more ribonucleotides, preferably more than three. Its exact size will depend upon many factors which. in turn, depend upon the ultimate function and use of the oligonucleotide.

The term "primer" as used herein refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product. which is complementary to a nucleic acid strand, is induced, i.e.. in the presence of nucleotides and an inducing agent such as a DNA polymerase and at a suitable temperature and pH. The primer may be either single-stranded or double-stranded and must be sufficiently long to prime the synthesis of the desired extension product in the presence of the inducing agent. The exact length of the primer will depend upon many factors, including temperature, source of primer and use of the method. For example, for diagnostic applications, depending on the complexity of the target sequence. the oligonucleotide primer typically contains 15-25 or more nucleotides, although it may contain fewer nucleotides.

The primers herein are selected to be "substantially" complementary to different strands of a particular target DNA sequence. This means that the primers must be sufficiently complementary to hybridize with their respective strands. Therefore, the primer sequence need not reflect the exact sequence of the template. For example, a non-complementary nucleotide fragment may be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the primer, provided that the primer sequence has sufficient complementarity with the sequence of the strand

to hybridize therewith and thereby form the template for the synthesis of the extension product.

As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to bacterial enzymes, each of which cut double-stranded DNA at or near a specific nucleotide sequence.

A cell has been "transformed" by exogenous or heterologous DNA when such DNA has been introduced inside the cell. The transforming DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming DNA may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the transforming DNA. A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

Two DNA sequences are "substantially homologous" when at least about 75% (preferably at least about 80%, and most preferably at least about 90 or 95%) of the nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Maniatis et al., *supra*; DNA Cloning, Vols. I & II, *supra*; Nucleic Acid Hybridization, *supra*.

It should be appreciated that also within the scope of the present invention are DNA sequences encoding eEF-2 kinase which code for a protein having the same amino acid sequence as SEQ ID NO: 2, 4, and 10, but which are degenerate to SEQ ID NO: 1, 3, and 9. By "degenerate to" is meant that a different three-letter codon is used to specify a particular amino acid. It is well known in the art that the following codons can be used interchangeably to code for each specific amino acid:

Phenylalanine (Phe or F)	UUU or UUC
Leucine (Leu or L)	UUA or UUG or CUU or CUC or CUA or CUG
Isoleucine (Ile or I)	AUU or AUC or AUA
Methionine (Met or M)	AUG
Valine (Val or V)	GUU or GUC of GUA or GUG
Serine (Ser or S)	UCU or UCC or UCA or UCG or AGU or AGC
Proline (Pro or P)	CCU or CCC or CCA or CCG
Threonine (Thr or T)	ACU or ACC or ACA or ACG
Alanine (Ala or A)	GCU or GCG or GCA or GCG
Tyrosine (Tyr or Y)	UAU or UAC
Histidine (His or H)	CAU or CAC
Glutamine (Gln or Q)	CAA or CAG
Asparagine (Asn or N)	AAU or AAC
Lysine (Lys or K)	AAA or AAG
Aspartic Acid (Asp or D)	GAU or GAC
Glutamic Acid (Glu or E)	GAA or GAG
Cysteine (Cys or C)	UGU or UGC
Arginine (Arg or R)	CGU or CGC or CGA or CGG or AGA or AGG
Glycine (Gly or G)	GGU or GGC or GGA or GGG
Tryptophan (Trp or W)	UGG
Termination codon	UAA (ochre) or UAG (amber) or UGA (opal)

It should be understood that the codons specified above are for RNA sequences. The corresponding codons for DNA have a T substituted for U.

Mutations can be made in SEQ ID NO: 1, 3, and 9 such that a particular codon is changed to a codon which codes for a different amino acid. Such a mutation is generally made by making the fewest nucleotide changes possible. A substitution mutation of this sort can be made to change an amino acid in the resulting protein in a non-conservative manner (*i.e.*, by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to another grouping) or in a conservative manner (*i.e.*, by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to the same grouping). Such a conservative change generally leads to less change in the structure and function of the resulting protein. A non-conservative change is more likely to alter the structure, activity or function of the resulting protein. The present invention should be considered to include sequences containing conservative changes which do not significantly alter the activity or binding characteristics of the resulting protein.

The following is one example of various groupings of amino acids:

<u>Amino acids with nonpolar R groups</u>

Alanine
Valine
Leucine
Isoleucine
Proline
Phenylalanine
Tryptophan
Methionine

Amino acids with uncharged polar R groups

Glycine
Serine
Threonine
Cysteine
Tyrosine
Asparagine
Glutamine

Amino acids with charged polar R groups (negatively charged at pH 6.0)

Aspartic acid
Glutamic acid

Basic amino acids (positively charged at pH 6.0)

Lysine
Arginine
Histidine (at pH 6.0)

Another grouping may be those amino acids with phenyl groups:

Phenylalanine
Tryptophan
Tyrosine

Another grouping may be according to molecular weight (i.e., size of R groups):

Glycine	75

Alanine	89
Serine	105
Proline	115
Valine	117
Threonine	119
Cysteine	121
Leucine	131
Isoleucine	131
Asparagine	132
Aspartic acid	133
Glutamine	146
Lysine	146
Glutamic acid	147
Methionine	149
Histidine (at pH 6.0)	155
Phenylalanine	165
Arginine	174
Tyrosine	181
Tryptophan	204

Particularly preferred substitutions are:

- Lys for Arg and vice versa such that a positive charge may be maintained;
- Glu for Asp and vice versa such that a negative charge may be maintained;
- Ser for Thr such that a free -OH can be maintained; and
- Gln for Asn such that a free NH_2 can be maintained.

Amino acid substitutions may also be introduced to substitute an amino acid with a particularly preferable property. For example, a Cys may be introduced a potential site for disulfide bridges with another Cys. A His may be introduced as a particularly "catalytic" site (*i.e.*, His can act as an acid or base and is the most common amino acid

in biochemical catalysis). Pro may be introduced because of its particularly planar structure, which induces β-turns in the protein's structure.

Two amino acid sequences are "substantially homologous" when at least about 70% of the amino acid residues (preferably at least about 80%. and most preferably at least about 90 or 95%) are identical. or represent conservative substitutions.

A "heterologous" region of the DNA construct is an identifiable segment of DNA within a larger DNA molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mammalian gene. the gene will usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. Another example of a heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., a cDNA where the genomic coding sequence contains introns, or synthetic sequences having codons different than the native gene). Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

An "antibody" is any immunoglobulin. including antibodies and fragments thereof, that binds a specific epitope. The term encompasses polyclonal. monoclonal, and chimeric antibodies, the last mentioned described in further detail in U.S. Patent Nos. 4,816,397 and 4,816,567.

An "antibody combining site" is that structural portion of an antibody molecule comprised of heavy and light chain variable and hypervariable regions that specifically binds antigen.

The phrase "antibody molecule" in its various grammatical forms as used herein contemplates both an intact immunoglobulin molecule and an immunologically active portion of an immunoglobulin molecule.

Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contains the paratope, including those portions known in the art as Fab, Fab', $F(ab')_2$ and F(v), which portions are preferred for use in the therapeutic methods described herein.

Fab and $F(ab')_2$ portions of antibody molecules are prepared by the proteolytic reaction of papain and pepsin, respectively, on substantially intact antibody molecules by methods that are well-known. See for example, U.S. Patent No. 4,342,566 to Theofilopolous et al. Fab' antibody molecule portions are also well-known and are produced from $F(ab')_2$ portions followed by reduction of the disulfide bonds linking the two heavy chain portions as with mercaptoethanol, and followed by alkylation of the resulting protein mercaptan with a reagent such as iodoacetamide. An antibody containing intact antibody molecules is preferred herein.

The phrase "monoclonal antibody" in its various grammatical forms refers to an antibody having only one species of antibody combining site capable of immunoreacting with a particular antigen. A monoclonal antibody thus typically displays a single binding affinity for any antigen with which it immunoreacts. A monoclonal antibody may therefore contain an antibody molecule having a plurality of antibody combining sites, each immunospecific for a different antigen; e.g., a bispecific (chimeric) monoclonal antibody.

The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human.

The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to prevent, and preferably reduce by at least about 30 percent, more preferably by at least 50 percent, most preferably by at least 90 percent, a clinically significant

change in the S phase activity of a target cellular mass, or other feature of pathology such as for example, elevated blood pressure, fever or white cell count as may attend its presence and activity.

A DNA sequence is "operatively linked" to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that DNA sequence. The term "operatively linked" includes having an appropriate start signal (e.g., ATG) in front of the DNA sequence to be expressed and maintaining the correct reading frame to permit expression of the DNA sequence under the control of the expression control sequence and production of the desired product encoded by the DNA sequence. If a gene that one desires to insert into a recombinant DNA molecule does not contain an appropriate start signal, such a start signal can be inserted in front of the gene.

The term "standard hybridization conditions" refers to salt and temperature conditions substantially equivalent to 5 x SSC and 65°C for both hybridization and wash. However, one skilled in the art will appreciate that such "standard hybridization conditions" are dependent on particular conditions including the concentration of sodium and magnesium in the buffer, nucleotide sequence length and concentration, percent mismatch, percent formamide, and the like. Also important in the determination of "standard hybridization conditions" is whether the two sequences hybridizing are RNA-RNA, DNA-DNA or RNA-DNA. Such standard hybridization conditions are easily determined by one skilled in the art according to well known formulae, wherein hybridization is typically 10-20°C below the predicted or determined T_m with washes of higher stringency, if desired.

In one aspect, the present invention relates to the identification of a new superfamily of protein kinases centered around eEF-2 kinase. Accordingly, it includes the DNA sequences coding for these family members. In addition, the invention also contemplates that each member of this new protein kinase superfamily has its own cognate

phosphorylation target. As specified *supra*, two of these targets are eEF-2 and MHC, which are phosphorylated by eEF-2 kinase and MHCK A, respectively.

In a particular embodiment, the present invention relates to phosphorylation target analogs, which are short peptide sequences derived from phosphorylation targets of this new superfamily of protein kinases centered around eEF-2 kinase. Specifically, it is contemplated that these peptide analogs will be instrumental in the development of high throughput screening assays to identify inhibitors of members of this new superfamily.

As overexpression of eEF-2 kinase has been associated with a variety of cancers and other hyperproliferitive pathologies (discussed *supra*), the invention also includes assay systems for the screening of potential drugs effective at inhibiting eEF-2 kinase activity. It is contemplated that any of the recited assays can be automated using technology that is standard to the skilled artisan.

As stated above, the present invention also relates to a recombinant DNA molecule or cloned gene, or a degenerate variant thereof, which encodes a eEF-2 kinase, or a fragment thereof, that possesses a molecular weight of about 100 kD and an amino acid sequence set forth in Figure 5 (SEQ ID NO: 2, 4, and 10); preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding the 100 kD eEF-2 kinase has a nucleotide sequence or is complementary to a DNA sequence shown in Figure 5 (SEQ ID NO: 1, 3, and 9).

Therapeutic possibilities are raised by the knowledge of the eEF-2 kinase sequence and the existence of peptide analogs that can act as phosphorylation targets for the kinase. Accordingly, it is contemplated that sequences that are derived from the complement to the eEF-2 kinase mRNA sequence, and various modifications thereof, can act as potent antisense drugs that either inhibit expression in a competitive fashion, or, more effectively, by nuclease activity associated with the antisense drug that cleaves the eEF-2 kinase mRNA sequence, thus rendering it irreversibly inactive. Alternative therapeutics

are also contemplated that concern the use of peptides and peptide analogs representing portions of phosphorylation target amino acid sequences. It is envisioned that such peptide-based drugs would inhibit eEF-2 kinase activity on its native target, thus bypassing the cascade of events that would lead to malignant transformation.

The antisense or peptide-based drugs may be prepared in pharmaceutical compositions, with a suitable carrier and at a strength effective for administration by various means to a patient experiencing an adverse medical condition associated with specific malignancies for the treatment thereof. A variety of administrative techniques may be utilized, among them parenteral techniques such as subcutaneous. intravenous and intraperitoneal injections, catheterizations and the like. Average quantities of the antisense or peptide-based drugs may vary and in particular should be based upon the recommendations and prescription of a qualified physician or veterinarian.

Also, antibodies including both polyclonal and monoclonal antibodies, and drugs that modulate the production or activity of eEF-2 kinase may possess certain diagnostic applications and may, for example, be utilized for the purpose of detecting and/or measuring levels of eEF-2 kinase. It is anticipated that further experimentation will reveal a prognostic correlation between eEF-2 kinase levels and the prediction and or progression of certain malignancies associated with carcinoma. For example, eEF-2 kinase may be used to produce both polyclonal and monoclonal antibodies to themselves in a variety of cellular media, by known techniques such as the hybridoma technique utilizing, for example. fused mouse spleen lymphocytes and myeloma cells. Likewise, small molecules that mimic or antagonize the activity of eEF-2 kinase of the invention may be discovered or synthesized, and may be used in diagnostic and/or therapeutic protocols.

The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal, antibody-producing cell lines can also be created by techniques other than fusion, such as direct transformation of B lymphocytes with oncogenic DNA, or

transfection with Epstein-Barr virus. See, e.g., M. Schreier et al., "Hybridoma Techniques" (1980); Hammerling et al., "Monoclonal Antibodies And T-cell Hybridomas" (1981); Kennett et al., "Monoclonal Antibodies" (1980); see also U.S. Patent Nos. 4,341,761; 4,399,121; 4.427,783; 4.444,887; 4,451,570; 4,466,917; 4,472,500; 4,491,632; 4,493,890.

Panels of monoclonal antibodies produced against eEF-2 kinase peptides can be screened for various properties; *i.e.*, isotype, epitope, affinity, etc. Of particular interest are monoclonal antibodies that neutralize the activity of eEF-2 kinase. Such monoclonals can be readily identified in eEF-2 kinase activity assays. High affinity antibodies are also useful when immunoaffinity purification of native or recombinant eEF-2 kinase is desired.

Preferably, the anti-eEF-2 kinase antibody used in the diagnostic methods of this invention is an affinity purified polyclonal antibody. More preferably, the antibody is a monoclonal antibody (mAb). In addition, it is preferable for the anti-eEF-2 kinase antibody molecules used herein be in the form of Fab, Fab', $F(ab')_2$ or F(v) portions of whole antibody molecules.

As suggested earlier, the diagnostic method of the present invention comprises examining a cellular sample or medium by means of an assay including an effective amount of an antagonist to eEF-2 kinase, such as an anti-eEF-2 kinase antibody, preferably an affinity-purified polyclonal antibody, and more preferably a mAb. In addition, it is preferable for the anti-eEF-2 kinase antibody molecules used herein be in the form of Fab, Fab', $F(ab')_2$ or F(v) portions or whole antibody molecules. As previously discussed, patients capable of benefiting from this method include those suffering from cancer, a pre-cancerous lesion, a viral infection or other like pathological derangement. Methods for isolating the eEF-2 kinase and inducing anti-eEF-2 kinase antibodies and for determining and optimizing the ability of anti-eEF-2 kinase antibodies to assist in the examination of the target cells are all well-known in the art.

Methods for producing polyclonal anti-polypeptide antibodies are well-known in the art. See U.S. Patent No. 4,493,795 to Nestor et al. A monoclonal antibody, typically containing Fab and/or $F(ab')_2$ portions of useful antibody molecules, can be prepared using the hybridoma technology described in *Antibodies - A Laboratory Manual*, Harlow and Lane, eds., Cold Spring Harbor Laboratory, New York (1988), which is incorporated herein by reference.

Splenocytes are typically fused with myeloma cells using polyethylene glycol (PEG) 6000. Fused hybrids are selected by their sensitivity to HAT. Hybridomas producing a monoclonal antibody useful in practicing this invention are identified by their ability to immunoreact with the present eEF-2 kinase and their ability to inhibit specified eEF-2 kinase activity in target cells.

A monoclonal antibody useful in practicing the present invention can be produced by initiating a monoclonal hybridoma culture comprising a nutrient medium containing a hybridoma that secretes antibody molecules of the appropriate antigen specificity. The culture is maintained under conditions and for a time period sufficient for the hybridoma to secrete the antibody molecules into the medium. The antibody-containing medium is then collected. The antibody molecules can then be further isolated by well-known techniques.

Media useful for the preparation of these compositions are both well-known in the art and commercially available and include synthetic culture media, inbred mice and the like. An exemplary synthetic medium is Dulbecco's minimal essential medium (DMEM; Dulbecco et al., *Virol.* **8**:396 (1959)) supplemented with 4.5 gm/l glucose, 20mM glutamine, and 20% fetal calf serum. An exemplary inbred mouse strain is the Balb/c.

Methods for producing monoclonal anti-eEF-2 kinase antibodies are also well-known in the art. See Niman et al., *Proc. Natl. Acad. Sci. USA*, **80**:4949-4953 (1983). Typically, the present eEF-2 kinase or a peptide analog is used either alone or conjugated to an

immunogenic carrier. as the immunogen in the before described procedure for producing anti-eEF-2 kianse monoclonal antibodies. The hybridomas are screened for the ability to produce an antibody that immunoreacts with the eEF-2 kinase peptide analog and the present eEF-2 kinase.

The present invention further contemplates therapeutic compositions useful in practicing the therapeutic methods of this invention. A subject therapeutic composition includes, in admixture, a pharmaceutically acceptable excipient (carrier) and one or more of an anti-eEF-2 kinase antibody, peptide analog capable of competing for phosphorylation of eEF-2 by eEF-2 kinase, antisense drug against eEF-2 kinase mRNA, or any other compound that is found to inhibit eEF-2 kinase activity. In a preferred embodiment, the composition comprises an antigen capable of modulating the activity of eEF-2 kinase within a target cell.

The preparation of therapeutic compositions which contain polypeptides, analogs or active fragments as active ingredients is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions, however, solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents which enhance the effectiveness of the active ingredient.

A polypeptide, analog or active fragment can be formulated into the therapeutic composition as neutralized pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide or antibody molecule) and which are formed with inorganic acids such as,

for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The therapeutic polypeptide-, analog- or active fragment-containing compositions are conventionally administered intravenously, as by injection of a unit dose, for example. The term "unit dose" when used in reference to a therapeutic composition of the present invention refers to physically discrete units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; *i.e.*, carrier, or vehicle.

The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the subject's immune system to utilize the active ingredient, and degree of inhibition or neutralization of eEF-2 kinase activity desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosages may range from about 0.1 to 20, preferably about 0.5 to about 10, and more preferably one to several, milligrams of active ingredient per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusion sufficient to maintain concentrations of ten nanomolar to ten micromolar in the blood are contemplated.

Formulations

Intravenous Formulation I

Ingredient	mg/ml
cefotaxime	250.0
antibody, peptide, antisense drug, or other compound	10.0
dextrose USP	45.0
sodium bisulfite USP	3.2
edetate disodium USP	0.1
water for injection q.s.a.d.	1.0 ml

Intravenous Formulation II

Ingredient	mg/ml
ampicillin	250.0
antibody, peptide, antisense drug, or other compound	10.0
sodium bisulfite USP	3.2
disodium edetate USP	0.1
water for injection q.s.a.d.	1.0 ml

Intravenous Formulation III

Ingredient	mg/ml
gentamicin (charged as sulfate)	40.0
antibody, peptide, antisense drug, or other compound	10.0
sodium bisulfite USP	3.2
disodium edetate USP	0.1
water for injection q.s.a.d.	1.0 ml

Intravenous Formulation IV

Ingredient	mg/ml
antibody, peptide, antisense drug, or other compound	10.0
dextrose USP	45.0
sodium bisulfite USP	3.2
edetate disodium USP	0.1

water for injection q.s.a.d. 1.0 ml

As used herein, "pg" means picogram. "ng" means nanogram, "ug" or "μg" mean microgram, "mg" means milligram, "ul" or "μl" mean microliter, "ml" means milliliter, "l" means liter.

Another feature of this invention is the expression of the DNA sequences disclosed herein. As is well known in the art, DNA sequences may be expressed by operatively linking them to an expression control sequence in an appropriate expression vector and employing that expression vector to transform an appropriate unicellular host.

Such operative linking of a DNA sequence of this invention to an expression control sequence, of course, includes, if not already part of the DNA sequence, the provision of an initiation codon. ATG, in the correct reading frame upstream of the DNA sequence.

A wide variety of host/expression vector combinations may be employed in expressing the DNA sequences of this invention. Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences. Suitable vectors include derivatives of SV40 and known bacterial plasmids, e.g., *E. coli* plasmids col El, pCR1, pBR322, pMB9 and their derivatives, plasmids such as RP4; phage DNAS, e.g., the numerous derivatives of phage λ, e.g., NM989, and other phage DNA, e.g., M13 and filamentous single stranded phage DNA; yeast plasmids such as the 2μ plasmid or derivatives thereof; vectors useful in eukaryotic cells, such as vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and phage DNAs, such as plasmids that have been modified to employ phage DNA or other expression control sequences; and the like.

Any of a wide variety of expression control sequences -- sequences that control the expression of a DNA sequence operatively linked to it -- may be used in these vectors to express the DNA sequences of this invention. Such useful expression control sequences

include, for example, the early or late promoters of SV40, CMV, vaccinia, polyoma or adenovirus, the *lac* system, the *trp* system, the *TAC* system, the *TRC* system, the *LTR* system, the major operator and promoter regions of phage λ, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase (e.g., Pho5), the promoters of the yeast α-mating factors, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof.

A wide variety of unicellular host cells are also useful in expressing the DNA sequences of this invention. These hosts may include well known eukaryotic and prokaryotic hosts, such as strains of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, fungi such as yeasts, and animal cells, such as CHO, R1.1, B-W and L-M cells, African Green Monkey kidney cells (e.g., COS 1, COS 7, BSC1, BSC40, and BMT10), insect cells (e.g., Sf9), and human cells and plant cells in tissue culture.

It will be understood that not all vectors, expression control sequences and hosts will function equally well to express the DNA sequences of this invention. Neither will all hosts function equally well with the same expression system. However, one skilled in the art will be able to select the proper vectors, expression control sequences, and hosts without undue experimentation to accomplish the desired expression without departing from the scope of this invention. For example, in selecting a vector, the host must be considered because the vector must function in it. The vector's copy number, the ability to control that copy number, and the expression of any other proteins encoded by the vector, such as antibiotic markers, will also be considered.

In selecting an expression control sequence, a variety of factors will normally be considered. These include, for example, the relative strength of the system, its controllability, and its compatibility with the particular DNA sequence or gene to be expressed, particularly as regards potential secondary structures. Suitable unicellular hosts will be selected by consideration of, e.g., their compatibility with the chosen vector,

their secretion characteristics, their ability to fold proteins correctly, and their fermentation requirements, as well as the toxicity to the host of the product encoded by the DNA sequences to be expressed, and the ease of purification of the expression products.

Considering these and other factors, a person skilled in the art will be able to construct a variety of vector/expression control sequence/host combinations that will express the DNA sequences of this invention on fermentation or in large scale animal culture.

The present invention extends to the preparation of antisense oligonucleotides and ribozymes that may be used to interfere with the expression of the eEF-2 kinase gene at the translational level. This approach utilizes antisense nucleic acid and ribozymes to block translation of a specific mRNA, either by masking that mRNA with an antisense nucleic acid or cleaving it with a ribozyme.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule. (See Weintraub, 1990; Marcus-Sekura, 1988.) In the cell, they hybridize to that mRNA, forming a double stranded molecule. The cell does not translate an mRNA in this double-stranded form. Therefore, antisense nucleic acids interfere with the expression of mRNA into protein. Oligomers of about fifteen nucleotides and molecules that hybridize to the AUG initiation codon will be particularly efficient, since they are easy to synthesize and are likely to pose fewer problems than larger molecules when introducing them into eEF-2 kinase-producing cells. Antisense methods have been used to inhibit the expression of many genes *in vitro* (Marcus-Sekura, 1988; Hambor et al., 1988).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single stranded RNA molecules in a manner somewhat analogous to DNA restriction endonucleases. Ribozymes were discovered from the observation that certain mRNAs have the ability to excise their own introns. By modifying the nucleotide sequence of

these RNAs, researchers have been able to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, 1988.). Because they are sequence-specific, only mRNAs with particular sequences are inactivated.

Investigators have identified two types of ribozymes, *Tetrahymena*-type and "hammerhead"-type. (Hasselhoff and Gerlach, 1988) *Tetrahymena*-type ribozymes recognize four-base sequences, while "hammerhead"-type recognize eleven- to eighteen-base sequences. The longer the recognition sequence, the more likely it is to occur exclusively in the target mRNA species. Therefore, hammerhead-type ribozymes are preferable to *Tetrahymena*-type ribozymes for inactivating a specific mRNA species, and eighteen base recognition sequences are preferable to shorter recognition sequences.

The DNA sequences described herein may thus be used to prepare antisense molecules against, and ribozymes that cleave mRNAs for eEF-2 kinase .

The present invention also relates to a variety of diagnostic applications, including methods for detecting and quantifying the levels of eEF-2 kinase. As mentioned earlier, eEF-2 kinase can be used to produce antibodies to itself by a variety of known techniques, and such antibodies could then be isolated and utilized as in tests for the presence and levels of eEF-2 kinase activity in suspect target cells.

As described in detail above, antibody(ies) to eEF-2 kinase can be produced and isolated by standard methods including the well known hybridoma techniques. For convenience, the antibody(ies) to eEF-2 kinase will be referred to herein as Ab_1 and antibody(ies) raised in another species as Ab_2.

The presence and levels of eEF-2 kinase in cells can be ascertained by the usual immunological procedures applicable to such determinations. A number of useful procedures are known. Three such procedures which are especially useful, utilize either eEF-2 kinase labeled with a detectable label, antibody Ab_1 labeled with a detectable label,

or antibody Ab_2 labeled with a detectable label. The procedures may be summarized by the following equations wherein the asterisk indicates that the particle is labeled, and "~" stands for eEF-2 kinase:

A. $\sim^* + Ab_1 = \sim^*Ab_1$

B. $\sim + Ab^* = \sim Ab_1{}^*$

C. $\sim + Ab_1 + Ab_2{}^* = \sim Ab_1Ab_2{}^*$

The procedures and their application are all familiar to those skilled in the art and accordingly may be utilized within the scope of the present invention. The "competitive" procedure, Procedure A, is described in U.S. Patent Nos. 3,654,090 and 3,850,752. Procedure C, the "sandwich" procedure, is described in U.S. Patent Nos. RE 31,006 and 4,016,043. Still other procedures are known such as the "double antibody," or "DASP" procedure.

In each instance, eEF-2 kinase forms complexes with one or more antibody(ies) or binding partners and one member of the complex is labeled with a detectable label. The fact that a complex has formed and, if desired, the amount thereof, can be determined by known methods applicable to the detection of labels.

It will be seen from the above, that a characteristic property of Ab_2 is that it will react with Ab_1. This is because Ab_1 raised in one mammalian species has been used in another species as an antigen to raise the antibody Ab_2. For example, Ab_2 may be raised in goats using rabbit antibodies as antigens. Ab_2 therefore would be anti-rabbit antibody raised in goats. For purposes of this description and claims, Ab_1 will be referred to as a primary or anti-eEF-2 kinase antibody, and Ab_2 will be referred to as a secondary or anti-Ab_1 antibody.

The labels most commonly employed for these studies are radioactive elements, enzymes, chemicals which fluoresce when exposed to ultraviolet light, and others.

A number of fluorescent materials are known and can be utilized as labels. These include, for example, fluorescein, rhodamine, auramine, Texas Red, AMCA blue and Lucifer Yellow. A particular detecting material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate.

eEF-2 kinase can also be labeled with a radioactive element or with an enzyme. The radioactive label can be detected by any of the currently available counting procedures. The preferred isotope may be selected from ^{3}H, ^{14}C, ^{32}P, ^{33}P, ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{90}Y, ^{125}I, ^{131}I, and ^{186}Re.

Enzyme labels are likewise useful, and can be detected by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques. The enzyme is conjugated to the selected particle by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like. Many enzymes which can be used in these procedures are known and can be utilized. The preferred are peroxidase, ß-glucuronidase, ß-D-glucosidase, ß-D-galactosidase, urease, glucose oxidase plus peroxidase and alkaline phosphatase. U.S. Patent Nos. 3,654,090; 3,850,752; and 4,016.043 are referred to by way of example for their disclosure of alternate labeling material and methods.

A particular assay system developed and utilized in accordance with the present invention, is known as a receptor assay. In a receptor assay, the material to be assayed is appropriately labeled and then certain cellular test colonies are inoculated with a quantity of both the labeled and unlabeled material after which binding studies are conducted to determine the extent to which the labeled material binds to the cell receptors. In this way, differences in affinity between materials can be ascertained.

Accordingly, a purified quantity of the eEF-2 kinase may be radiolabeled and combined, for example, with antibodies or other inhibitors thereto, after which binding studies would be carried out. Solutions would then be prepared that contain various quantities

of labeled and unlabeled uncombined eEF-2 kinase, and cell samples would then be inoculated and thereafter incubated. The resulting cell monolayers are then washed, solubilized and then counted in a gamma counter for a length of time sufficient to yield a standard error of <5%. These data are then subjected to Scatchard analysis after which observations and conclusions regarding material activity can be drawn. While the foregoing is exemplary, it illustrates the manner in which a receptor assay may be performed and utilized, in the instance where the cellular binding ability of the assayed material may serve as a distinguishing characteristic.

In accordance with the above, an assay system for screening potential drugs effective to modulate the activity of eEF-2 kinase may be prepared. The eEF-2 kinase may be introduced into a test system, and the prospective drug may also be introduced into the resulting cell culture, and the culture thereafter examined to observe any changes in the eEF-2 kinase activity of the cells, due either to the addition of the prospective drug alone, or due to the effect of added quantities of the known eEF-2 kinase. Alternatively, these assays can be carried out in a purely *in vitro* fashion as discussed below.

The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

EXPERIMENTAL DETAILS SECTION

EXAMPLE 1

Peptide Sequencing. eEF-2 kinase from rabbit reticulocyte lysate was purified as described (Hait et al., (1996) *FEBS Lett.* 397:55-60). Peptides were generated from the nitrocellulose-bound 103-kDa eEF-2 kinase protein by *in situ* tryptic digestion (Erdjument-Bromage et al., (1994) *Protein Sci.* 3:2435-2446) and fractionated by reverse-phase HPLC (Elicone et al., (1994) *J. Chromatogr.* 676:121-137) using a 1.0 mm Reliasil C18 column. Selected peak fraction were then analyzed by a combination of automated

Edman sequencing and matrix-assisted laser-desorption time-of-flight mass spectrometry (Erdjument-Bromage et al., (1994)). The peptide sequences provided an essential lead into the cloning of eEF-2 kinase from human, mouse, rat, and *Caenorhabditis elegans*.

EXAMPLE 2

Molecular Cloning of cDNAs Encoding *C. elegans*, Mouse, Rat, and Human eEF-2 Kinases. To clone the cDNA for *C. elegans* eEF-2 kinase, oligonucleotide primers were designed based on the amino and carboxy termini of the predicted gene product from F42A10.4. Reverse transcriptase-PCR (RT-PCR) was performed using these primers and total RNA from *C. elegans* (a gift form Monica Driscoll, Rutgers University). A single PCR product of ~2.3 kb was obtained and gel-purified using a gel extraction kit (Qiagen, Chatsworth, CA). The fragment was ligated into vector pCR2.1 using the TA cloning kit (Invitrogen, Sorrento Valley, CA), and then transformed into *Escherichia coli*. Plasmid DNA was purified, and restriction analysis used to verify the orientation of the coding sequence with respect to the T7 promoter. Two clones (*Cefk-1 and Cefk-2, C. elegans* eEF-2 kinase isoforms 1 and 2) were chosen and sequenced using a Li-Cor (Lincoln, NE) Long Read IR model 400L Automated DNA Sequencer. Analysis revealed that the two clones were identical except for a deletion of 24 bp in *Cefk-2* which corresponds to exon 4 and probably represents an alternatively spliced form.

To clone the mouse eEF-2 kinase, degenerate primers were designed based on the amino acid sequence of two peptides from rabbit eEF-2 kinase (LTPQAFSHFTFER (SEQ ID NO: 21) and LANXYYEKAE (SEQ ID NO: 22)): primer A, CA(G/A)GC(C/G/T/A)TT(C/T)(T/A)(C/G)(T/CCA(C/T)TT(C/T)AC(C/G/T/A)TT(C/T)GA(G/A(C/A)G (SEQ ID NO: 23); and primer B, TC(C/G/T/A)GC(C/T)TT(C/T)TC(G/A)TA(G/A)TA(C/T)TT(G/A)TT(C/G/A/T)GC (SEQ ID NO: 24). RT-PCR was performed using primers A and B and poly(A)$^+$ RNA from mouse spleen (CLONTECH). A single PCR product (~1.6 kb) was cloned into pCR2.1 (Invitrogen) and sequenced. Using sequence information form these mouse eEF-2 kinase cDNA fragments, new primers were designed for 5′ rapid amplification of

cDNA ends (RACE) and 3′ RACE to obtain full-length mouse eEF-2 kinase cDNA. 5′ RACE and 3′ RACE were performed using Marathon-Ready mouse spleen cDNA (CLONTECH). This was carried out according to the manufacturer's instructions using the primers AP1 and C (TACAATCAGCTGATGACCAGAACGCTC) (SEQ ID NO: 25) 5′ antisense, or D (GGATTTGGACTGGACAAGAACCCCC) (SEQ ID NO: 19) 3′ sense.

To clone rat eEF-2 kinases, PCR was performed on a rat PC12 cDNA library cloned in λGT10 (CLONTECH) using primer B and vector primers. A 700-bp fragment was specifically amplified. The fragment was cloned into pCR2.1 (Invitrogen) and sequenced. This 700-bp fragments was radiolabeled and used to probe the same PC12 cDNA library (600.000 plaques). Fourteen positives were obtained in the initial screening. Five plaques were chosen for further analysis and sequencing based on insert sizes that ranged from 1.4 to 2.0 kb.

Recently, eEF-2 kinase from rabbit reticulocyte lysate was purified to near homogeneity (Hait et al., (1996)). This enabled determination of its partial amino acid sequence (see EXAMPLE 1). Two peptide sequences (LTPQAFSHFTFER and LANXYYEKAE) were compared with entries in a nonredundant database using the National Center for Biotechnology Information BLAST program (Altschul et al., (1990) *J. Mol. Biol.* 215:403-410). Matches were found with a *C. elegans* hypothetical protein (F42A10.4; GenBank accession number U10414). This sequence was obtained from the *C. elegans* genome sequencing project and is located on chromosome III (Wilson et al., (1994) *Nature* 368:32-38). The 100% identity between the sequenced peptides and the *C. elegans* protein, as well as the fact that the predicted molecular weight of the *C. elegans* protein is similar to that of eEF-2 kinase, suggested that this gene encoded eEF-2 kinase. The full-length cDNA by RT-PCR using *C. elegans* total RNA was cloned. Several clones were isolated and sequenced. *Cefk-1* has six of the predicted exons and encodes 768 amino acids. *Cefk-2* represents an alternatively spliced form that has five exons; it is missing amino acids 625-632 that correspond to exon four.

As is demonstrated in EXAMPLE 3, *Cefk-1* and *Cefk-2* have eEF-2 kinase activity when expressed in cell-free system using a wheat germ extract coupled transcription/translation system.

To determine the amino acid sequence of mammalian eEF-2 kinase, the cDNA of mouse eEF-2 kinase was cloned and sequenced. Since the sequenced peptides from rabbit eEF-2 were 100% identical to *C. elegans* eEF-2 kinase, then the two peptides should also match the sequence of mouse eEF-2 kinase. Degenerate primers were designed based on the amino acid sequence of the peptides and were used to perform RT-PCR on mouse spleen poly(A)$^+$ mRNA. A single PCR product of ~1.6 kb was obtained and sequenced. To obtain the full-length cDNA, 5′ RACE and 3′ RACE were performed using mouse spleen cDNA. The full-length cDNA, which encodes 724 amino acids, was expressed in a cell-free coupled transcription/translation system. A single translation product with an apparent molecular weight of 100 kDa was obtained (Figure 2).

cDNA for rat eEF-2 kinase using a fragment of mouse eEF-2 kinase cDNA to probe a PC12 cDNA library was cloned and sequenced. However, after this work was completed, a paper describing the cloning of eEF-2 from rat skeletal muscle was published (Redpath et al., (1996) *J. Biol. Chem.* 271:17547-17554) and the reported sequence appears to be identical to the eEF-2 kinase sequence from PC12 cells. Like the mouse eEF-2 kinase, the rat eEF-2 kinase cDNA encodes a 724-amino acid protein.

The human eEF-2 kinase cDNA was cloned and sequenced. RT-PCR was performed on poly(A)$^+$ mRNA from the human glioma cell line T98G using 20'mer primers corresponding to the 5′ and 3′ ends of the mouse eEF-2 kinase coding region. The human eEF-2 kinase cDNA encodes a 725 amino acid protein.

EXAMPLE 3

Expression of eEF-2 Kinase From *C. elegans*, Mouse, Rat, and Human in a Cell-Free System. Plasmid DNA from clones *Cefk-1*, *Cefk-2*, as well as mouse and human eEF-2 kinase cDNA were used in the TNT wheat germ extract coupled transcription/translation system (Promega). [^{35}S]Methionine-labeled products were then analyzed by SDS/PAGE. The reaction mixture (50 μl total volume) contained 1 μg of plasmid DNA and 26 μCi of [^{35}S]methionine (specific activity = 1175.0 Ci/mmol; 1 Ci = 37 GBq). Other components were added to the reaction mixture according to the manufacturer's protocol. The reaction mixture was incubated for 1.5 h at 30°C and terminated by incubation on ice. A 10 μl aliquot of the reaction mixture was mixed with 2 μl of 5X Laemmli buffer and boiled for 5 min. Samples were analyzed by SDS/PAGE on 8% gels and autoradiography.

The remainder of the transcription/translation reaction was diluted 4-fold with buffer A (20 mM Tris-HCl, pH 7.4/1 mM $MgCl_2$/10% glycerol/7 mM 2-mercaptoethanol) and applied to a HR5/5 Mono Q column (Pharmacia) equilibrated with buffer A. The column was developed with 20 column volumes of a 50-600 mM KCl linear gradient to buffer A.

To assay for eEF-2 kinase activity, 5 μl from each fraction was added to a reaction mixture (40 μl) containing 50 mM Hepes-KOH (ph. 7.4) 10 mM magnesium acetate, 0.1 mM $CaCl_2$, 5 mM dithiothreitol, 50 μM ATP, 2 μCi [γ-^{32}P]ATP, 0.6 μg calmodulin, and 0.5 μg rabbit reticulocyte eEF-2. Reactions were incubated at 30°C for 2 min and were terminated by adding 20 μl of 3X Laemmli sample buffer. Samples were boiled for 5 min and proteins separated by SDS/PAGE on 8% gels. Phosphoproteins were analyzed by autoradiography.

To determine whether Cefk-1 and Cefk-2 have eEF-2 kinase activity, cell-free coupled transcription/translation system were expressed. Translation of *Cefk-1* and *Cefk-2* produced products with an apparent molecular weight of 100 kDa (Figure 2), which is

slightly larger than the computer-predicted molecular weight of the protein but is identical to the molecular weight of a rabbit reticulocyte eEF-2 kinase as determined by SDS/PAGE. The translation products of the mixture of *Cefk-1* and *Cefk-2* are able to phosphorylate eEF-2 (Figure 3) and elute from a Mono Q column at the same position as endogenous *C. elegans* eEF-2 kinase (Figure 3A). The eEF-2 phosphorylation activity of the recombinant protein is Ca^{2+}/calmodulin-dependant (Figure 3C). The differences in the catalytic properties Cefk-1 and Cefk-2 isoforms are under current investigation.

Mouse and human eEF-2 kinase cDNAs were expressed in a coupled transcription/translation system and a product of ~100 kDa was obtained (Figure 2). As shown in Figure 3, the recombinant human eEF-2 kinase activity was strictly Ca^{2+}/calmodulin-dependant. The kinase activity was completely inhibited by the calmodulin antagonists trifluoperazine and *N*-(6-aminohexyl)-5-chloro-1-napthalene-sulfonamide. Human eEF-2 kinase in bacteria as a glutathione S-transferase fusion protein was expressed and demonstrated that the ability of the recombinant enzyme to phosphorylate eEF-2 and to undergo autophosphorylation are strictly calmodulin-dependent.

EXAMPLE 4

Analysis of Mouse eEF-2 Kinase mRNA Expression in Various Tissues. eEF-2 kinase and eEF-2 hybridizations were performed using a 1.6 kb *Eco*RI mouse cDNA fragment and a 2.6 kb *Eco*RI human cDNA fragment, respectively. cDNAs were labeled with [^{32}P]dCTP using the random-primed DNA labeling method (Feinberg and Vogelstein (1983) *Anal. Biochem.* 132:6-13). A multiple tissue Northern blot (CLONETECH) was prehybridized at 42°C for 16 h in a 50% formamide solution containing 10X Denhardt's, 5X SSPE, 2% SDS, and 100 μg/ml salmon sperm DNA. Hybridizations were completed in the same solution containing the ^{32}P-labeled probe (1 X 10^6 cpm/ml; specific activity, ~1 X 10^8 dpm/μg DNA) and 10% dextran sulfate at 42°C for 16 h. Blots were washed twice at room temperature (15 min) in 2X SSPE, 0.05%

SDS, and once at 50°C (15 min) in 0.5X SSPE. 0.5% SDS. RNA/cDNA hybrids were visualized by autoradiography.

Northern blot analysis shows that eEF-2 kinase is ubiquitously expressed in mouse tissues and is particularly abundant in skeletal muscle and heart (Figure 4). The abundance of eEF-2 kinase mRNA in muscle tissues may indicate that phosphorylation of eEF-2 is particularly important in muscle, or that there are additional substrates of eEF-2 kinase which are muscle-specific.

EXAMPLE 5

Lack of Homology of eEF-2 Kinase to Members of Eukaryotic Protein Kinase Superfamily. The alignment of the amino acid sequences of *C. elegans* and mammalian eEF-2 kinases is shown in Figure 5. Rat and mouse eEF-2 kinase are very similar being 97% identical and differing by only 23 amino acids. Human eEF-2 kinase is 90% identical to mouse and rat eEF-2 kinase. In contrast, *C. elegans* eEF-2 kinase is found to be only 40% identical to mammalian eEF-2 kinase.

According to the current classification, eEF-2 kinase belongs to the family of closely related calmodulin-dependent protein kinases. Surprisingly, upon analyzing eEF-2 kinase sequences, any homology to the other calmodulin-dependent kinases or to any other members of the protein kinase super-family was not found. The only motif which it shares with all other protein kinases is the GXGXXG motif (279-284 in *C. elegans* eEF-2 kinases; 295-300 in mouse eEF-2 kinase) which forms a glycine-rich loop and is part of the ATP-binding site. Comparison of mammalian and *C. elegans* eEF-2 kinase revealed only one extended region of homology that spans ~200 amino acids upstream of the GXGXXG motif. The high degree of similarity and the proximity to the nucleotide-binding site suggests that these 200 amino acids represent the catalytic domain. This region has a high degree of similarity and a portion of this region (amino acids 251-300 in mouse eEF-2 kinase) displays 75% identity to the catalytic domain of

MHCKA (see below), which also suggests that this is the catalytic domain. In the recently published rat eEF-2 kinase sequence [Redpath *et al.*, *J. Biol. Chem.* **271**: 17547-17554 (1996)], the catalytic domain was predicted to reside between amino acids 288 and 554 based on the homology with the catalytic domain of cAMP-dependant protein kinase (PKA). The results demonstrate that their prediction cannot be correct for several reasons. First, the homology of this region with PKA is not statistically significant. Second, this region is the least conserved between mammalian and *C. elegans* eEF-2 kinase. Finally, according to secondary structure predictions [made by Alexei V. Finkelstein. Institute of Protein Research. Russia using the ALB-GLOBULE program [Ptitsyn and Finkelstein, *Biopolymers* **22**:15-25 (1983)]], this region most likely has a distorted structure and contains almost no α-helices or β-strands, which are characteristic of a catalytic domain.

Because eEF-2 kinase is CA^{2+}/calmodulin-dependant, it should contain a calmodulin-binding domain, which is usually represented by an amphipathic α-helix. There are several regions that could possibly assume an amphipathic α-helical conformation. Further biochemical analysis is required to determine which of these is the calmodulin-binding domain.

In the C-terminal region, there is a short stretch of 22 amino acids which is 86% identical between mammalian and *C. elegans* eEF-2 kinase and is preceded by a longer region of weak homology. One of the possibilities is that it is that it is involved in oligomerization of the kinase. It was thought previously that eEF-2 kinase was an elongated monomer because it migrated during gel filtration as an ~150-kDa protein and migrated on SDS gels as a 105-kDa polypeptide [Ryazanov and Spirin, *Translational Regulation of Gene Expression*, Pienum, NY, Vol 2, pp 433-455 (1993); Abdelnajid *et al.*, *Int. J. Dev. Biol.*, 37:279-290 (1993)]. However, the molecular weight of a monomer of mammalian eEF-2 kinase based on the predicted sequence is just 82 kDa. Thus, it is possible that eEF-2 kinase is not a monomer but a responsible for dimerization. Interestingly, according to computer prediction using the COIL

program, this conserved region can form a coiled-coil. Formation of coiled-coil is often responsible for dimerization [Lupas, *Trends Biochem. Sci.*, **21**:375-382 (1996)].

EXAMPLE 6

Striking Homology Between eEF-2 Kinase and MHCK A from *Dictyostelium*. It was found that eEF-2 kinases is homologous to the central portion of the recently described MHCKA from *Dictyostelium* [Futey *et al.*, *J. Biol.. Chem.* **270:**523-529 (1995) see Figure 5]. The kinase was biochemically identified as a 130-kDa protein and has a demonstrated role in myosin assembly, both *in vitro* and *in vivo* [Futey *et al.*, 1995, *supra*]. As with eEF-2 kinase, MHCKA displays no region with detectable similarity to the conserved catalytic domains found in known eukaryotic protein kinases. Primary structure analysis of MHCKA revealed an amino-terminal domain with a probable coiled-coil structure, a central nonrepetitive domain, and a C-terminal domain consisting of seven WD repeats [Futey *et al.*, 1995, *supra*]. A fragment of the central nonrepetitive domain of MHCKA containing amino acids 552-841 was recently shown to represent the catalytic domain [Cote *et al.*, *J. Biol. Chem.* **272**:6846-6849 (1997)].

Because the catalytic domain of MHCKA and eEF-2 kinase have a high degree of similarity, the substrate specificity of these two kinases was assayed. Figure 6 shows that MHCK A cannot phosphorylate eEF-2, and likewise, rabbit eEF-2 kinase cannot use myosin heavy chains as a substrate. This demonstrated that each of these kinases is specific for their respective substrates.

EXAMPLE 7

eEF-2 Kinase and MHCK A Define a New Class of Protein Kinases. Members of the eukaryotic protein kinase superfamily are characterized by a conserved catalytic domain containing approximately 260 amino acids and is divided into twelve

subdomains [Hanks and Hunter, *FASEB J.*, **9**:576-596 (1996); Hardie and Hanks, *The Protein Kinase Facts Book,* Academic, London (1995), Taylor *et al.*, *Annu. Rev. Cell Biol.* **8**:429-462 (1992) Johnson *et al.*, *Cell.* **85**: 149-158 (1996)]. The three-dimensional structure of several protein kinases revealed that the catalytic domain consists of two lobes. The smaller N-terminal lobe, which has a twisted β-sheet structure, represents the ATP-binding domain. The larger C-terminal lobe, which is predominantly α-helical is involved in substrate binding. At the primary structure level, the only motif similar between eEF-2 kinase, MHCK A, and other protein kinases is the GXGXXG motif which forms the loop interacting directly with the phosphates of ATP [Hanks and Hunter, 1996, *supra*; Hardie and Hanks 1995, *supra*; Taylor *et al..*, *supra*]. In eukaryotic protein kinases, this motif is located at the very N terminus of the ATP-binding lobe of the catalytic domain. In contrast, in a eEF-2 kinase and MHCK A, this motif is close to the C terminus of the catalytic domain (see Figure 7). However, the overall topology of the ATP-binding subdomain of eEF-2 kinase and MHCK A can be similar to other protein kinases because the region upstream of the GXGXXG motif is strongly predicted to contain four or five β-strands and thus can form a twisted β-sheet.

However, the mechanism of ATP-binding to eEF-2 kinase is probably quite different in comparison to other conventional members of the eukaryotic protein kinase superfamily. In protein kinases, there is a conserved lysine residue, corresponding to Lys-72 in cAMP-dependant protein kinases which binds to the β- and γ-phosphates of ATP and is located at about 20 amino acids downstream of the GXGXXG motif. Analysis of eEF-2 kinase and MHCK A sequences revealed that there are no conserved lysine residues in the vicinity of the GXGXXG motif. There is another atypical protein kinase, BCR-ABLE, which does not contain this conserved lysine and it is proposed that it interacts with ATP *via* two cysteine residues [Maro and Witte, *Cell*, **67**:459-468 (1991)]. Interestingly, eEF-2 kinase and MHCK-A contain two conserved cysteine residues (Cys-313 and Cys-317 in mouse eEF-2 kinase) which are located near the GXGXXG motif and therefore might be involved in ATP binding. Thus the

mechanism of ATP-binding of eEF-2 kinase and MHCK A is different from other members of the protein kinase superfamily, but may be similar to that of the BCR-ABLE protein kinase.

The overall catalytic mechanism of eEF-2 kinase and MHCKA is probably also very different from other eukaryotic protein kinases. All members of the eukaryotic protein kinase superfamily contain a DXXXN motif in the catalytic loop and a DFG motif in the activation segment [Hanks and Hunter, 1996; *supra*, Hardie and Hanks 1995, *supra*; Taylor *et al.*, *supra;* Johnson *et al.*, 1996, *supra*]. These two motifs, which are directly involved in the catalysis of the protein phosphorylation reaction, are absent from the eEF-2 kinase and MHCK A catalytic domain.

It is not known whether there are other protein kinases which are structurally similar to eEF-2 kinase and MHCK A. An extensive search of the entire nonrestricted database of the National Center for Biotechnology Information using the BLAST program did not reveal any protein with a significant homology to the catalytic domain of eEF-2 kinase and MHCKA. A search of the Expressed Sequence Tag (EST) database revealed several ESTs from *C. elegans,* mouse and human which are essentially identical to portions of eEF-2 kinase cDNA sequences reported here. Interestingly, a search of the recently completed genome database of *Saccharomyces cerevisiae* did not reveal any protein with homology to eEF-2 kinase despite the fact that eEF-2 phosphorylation was reported in yeast (41).

Conclusion. Since the catalytic domains of eEF-2 kinase and MHCK A do not share homology with other known protein kinases, these two protein kinases establish the presence of a novel and widespread superfamily of eukaryotic protein kinases. Although the existence of several unusual protein kinases have been reported, to the knowledge, it was demonstrated for the first time the existence of a biochemically well-characterized and ubiquitous protein kinase that is structurally unrelated to other serine/threonine/tyrosine kinases. Contrary to the widely accepted belief that all

eukaryotic protein kinases evolved from a single ancestor, the results suggest that eukaryotic protein kinases appeared at least twice during the course of evolution. This also suggests that, in addition to the relatively well-characterized catalytic mechanism employed by members of eukaryotic serine/threonine/tyrosine protein kinase superfamily, there exists another mechanism of protein kinase superfamily, there exists another mechanism of protein phosphorylation. Further studies will reveal the molecular details of this mechanism and whether there are other protein kinases that phosphorylate their substrates using this mechanism.

EXAMPLE 8

Preparation of recombinant eEF-2 kinase fusion proteins with GST, 6xHis, and thioredoxin. Human eEF-2 kinase cDNA was cloned into three different expression vectors: pGEX-2T (Pharmacia Biotech, Piscataway, NJ); pRSET A (Invitrogen, Sorrento Valley, CA); and, pThioHisB (Invitrogen). After transformation into *Escherichia coli* strain BL21(DE3), transformants were cultured in LB broth containing 50 μg/ml ampicillin. When the cultured reached an A_{600} value of 0.7, isopropyl-β-thiogalactopyranoside (IPTG) was added to the bacterial cultures to a final concentration of 0.5 μM to induce expression. After three hours, the cultures were harvested by centrifugation, and the cells were then sonicated. After extract preparation and analysis by SDS-PAGE, it was found that all of the expressed tag forms of the eEF-2 kinase were in inclusion bodies. Inclusion bodies were precipitated, dissolved in 8.0 M urea, and dialyzed overnight against 20 mM Tris-HCl (ph. 7.0) buffer containing 100 mM NaCl and 4 mM β-mercaptoethanol. The refolded protein was analyzed by SDS-PAGE and assayed for the ability to phosphorylate eEF-2. All of the fusion eEF-2 kinase preparations were able to efficiently phosphorylate eEF-2.

EXAMPLE 9

eEF-2 Kinase Activity Assay Using a 16-Amino Acid Peptide Derived from Myosin Heavy Chain as the Phosphorylation Target. It was found that 16'mer peptide, RKKFGESEKTKTKEFL, can serve as a good substrate for eEF-2 kinase. (Note: circular

dichroism measurements indicated that this peptide is in an α-helical structure, and that amidation of the peptide further stabilizes the α-helical structure, resulting in stronger phosphoacceptor activity.) Since recombinant eEF-2 is impossible to overexpress, as discussed *supra*, and large amounts of the protein are required to for large scale screening assays, the discovery of a peptide (easily synthesized on a large scale) that exhibits the same phosphoacceptor activity as eEF-2 was the critical breakthrough that allows for the development of a variety of automated high throughput screening assays for screening drug candidates.

The basic assay is as follows: 0.2-10.0 μg of recombinant eEF-2 kinase (produced as described in EXAMPLE 6) is incubated with the 16'mer peptide (described above) in a buffer consisting of 12.5 mM Hepes-KOH (ph. 7.4), 2.5 mM magnesium acetate, 1.25 mM DTT, 25 μM $CaCl_2$, 0.05-2.5 μg calmodulin, 100 μM ATP, and 0.5 μCi [γ-^{33}P]ATP in a total volume of 5-250 μl. Samples are incubated at 30°C and aliquots can be withdrawn at various time points or at a single end point, and the reaction terminated by lowering the temperature (≤4°C). The aliquots are then spotted onto phosphocellulose paper (2 cm x 2 cm) and washed (4 x 4 min) with 75 mM phosphoric acid. The papers are then rinsed with 100% ethanol, dried, and then counted in a scintillation counter. The assay can be performed at various peptide concentrations, as was done in the experiment illustrated in Figure 8. Clearly for a high throughput drug screening assay, that would be amenable to automation, the assays would most likely be performed using one peptide concentration with increasing amounts of different drug (inhibitor) candidates, and the data collected at a single time point. The assay can be performed in any one of the following formats:

1. with [γ-^{32}P]ATP or [γ-^{33}P]ATP and then detected using either standard scintillation counting, or detected in the format of a homogeneous assay using a Scintillation Proximity Assay, described in detail in both the Amersham Product Catalog (1997), pp. 252-258, and U.S. patent number 4,568,649;

2. in any of a number of standard immunoassay formats using antibodies that are specific for the phosphorylated form of the 16'mer peptide. Detection would then be, as described in more detail *supra*, through the use of either isotopically- or nonisotopically-labeled antibodies, secondary antibodies, or 16'mer peptide.

EXAMPLE 10

Cancer cell killing by chemotherapeutic drugs requires protein synthesis, and can be blocked by cycloheximide

It has been well established that protein synthesis is required for apoptotic cell death induced by a variety of stimuli, including anticancer drugs. Inhibition of protein synthesis by cycloheximide prevent apoptotic cell death, and protects cancer cells from treatment with anticancer drugs. The exact molecular mechanism by which protein synthesis modulates cell death is unclear. The results demonstrated herein demonstrate that translational elongation factor-2 kinase (eEF-2 kinase) can be activated by a slight decrease in pH. Activation of this kinase leads to commonly observed during apoptotic cell death, activation of this kinase, and hence inhibition of protein synthesis during apoptosis, is expected to be anti-apoptotic. Because of the possible anti-apoptotic function of eEF-2 kinase, inhibition of eEF-2 kinase is expected to sensitize tumors to apoptotic cell death and anticancer drugs. Specifically, activation of eEF-2 kinase in hypoxic solid tumors (a low pH environment) is anti-apoptotic. Consequently, the specific inhibition of eEF-2 kinase can sensitize hypoxic solid tumors in apoptotic cell death and to the cytotoxic action of anticancer drugs.

One of the major obstacles in cancer therapy is the resistance of cancer cells to chemotherapeutic drugs. Of the many types of drug resistance, the most commonly addressed is multidrug resistance. Multidrug resistance is a particular phenotype which is characterized by an unusual resistance of cells to a variety of anticancer drugs with unrelated chemical structures. Among various mechanisms of multidrug resistance, the

most important and intensively studied is the multidrug resistance conferred by P-glycoprotein. According to the current model, P-glycoprotein protects cells by actively pumping drugs out of cells.

Recent evidence suggests that there is another type of drug resistance that involves inhibition of programmed cell death, or apoptosis. Due to intensive research in the past several years, it has become well established that anticancer drugs kill cells by inducing apoptosis. Apoptosis is an active process that is accompanied by activation of specific signal transduction pathways, and requires expression of specific proteins. Although there are a few exceptions, in most cases, it has been observed that inhibition of protein synthesis by cycloheximide can block cell death induced by anticancer drugs. Table 1 provides a list of examples where it was demonstrated that cycloheximde can prevent cytotoxicity of anticancer drugs.

Table 1: Examples where cycloheximide was shown to protect cells and tissues from cell death caused by anticancer drugs.

Drug	Cell Type	Reference
Topolsomerase I inhibitors		
Camptothecin	HL-60 (human promyelocyte)	5. Gong et al. (1993) *J. Cell Physiol.* **157**:263-270.
Camptothecin	Mouse thymocytes	6. Onishi et al., (1993) *Biochem. Biphys. Acta* **1175**: 147-154.
CPT-11	PLC (human hepatoma)	7. Suzuki & Kato (1996) *Exp. Cell Res.* **227**:154-159.
Topolsomerase II inhibitors		
Doxorubicin (adriamycin)	V79 (rodent fibroblasts0	8. Bonner & Lawrence (1989) *Int. J. Radiat. Oncol. Biol. Phys.* **16**:1209-1212.

Doxorubicin (adriamycin)	P388 (mouse leukemia): mice	9. Furusawa et al. (1995) *Biol. Pharm. Bull.* **18**:1367-1372.
Doxorubicin (adriamycin)	murine intestinal tract; mouse thymocytes	10. Thakkar & Potten (1992) *Biochem. Pharm.* **43**:1683-1691.
Etoposide	Balb/C 3T3 (mouse fibroblasts); CCRF-CEM (human lymphoblast cells); L1012 (mouse leukemia cells)	11. Chow et al. (1988) *Biochem. Pharm.* **37**1117-1122.
Etoposide	mouse thymocytes	12. Sun et al. (1994) *Biochem. Pharm.* **47**: 187-195.
Microtubule drugs		
Taxol	KB (human epidermoid carcinoma);	13. Ling et al. (1998) *Int. J. Cancer* **75**:925-932
	A549 (human lung adenocarcinoma) MCF-7 (human breast)	14. Liebman et al. (1994) *Anticancer Drugs* **5**:287-292
Vincristine	Ksu (human osteosarcoma)	15. Sakai et al. (1989) *Cancer Res.* **49**:1193-1196.
Vincristine	CHO strain AA8	16. Kung et al. (1990) *Cancer Res.* **50**:7307-7317.
Colchicine	Ksu	15. Sakai et al. (1989) *Cancer Res.* **49**:1193-1196
Other drugs		
Ara-C	rat intestine	17. Verbin et al. (1973) *Cancer Res.* **33**:2086-2093.
Nitrogen mustard	rat intestine	18. Lieberman et al. (1970) *Cancer Res.* **30**:942-951.
5-AZT	mouse thymocytes	19. Kizaki et al. (1993) *Immunopharm.* **25**:19-27.

Cisplatin	CHO	20. Barry et al. (1990) *Biochem. Pharm.* **40**:2353-2362
Methotrexate	mice	21. Panasci et al. (1982) *Cancer Lett.* **15**:81-86.

While most of these studies were performed on cells in culture, there is also evidence that protein synthesis is required for the cytotoxic action of anticancer drugs *in vivo*. It was demonstrated by Furusawa et al. (9) that, in mice, the toxicity of doxurbicin as well as its antitumor effects, can be effectively counteracted by injection of cycloheximide. In another study (21) it was found that injection of cycloheximide can significantly reduce the toxicity of methotrexate. Thus, inhibition of protein synthesis may be considered a mechanism that confers resistance to anticancer drugs.

Cellular mechanism of protein synthesis inhibition: elongation factor-2 phosphorylation: It appears that, although eEF-2 kinase is strictly Ca^{2+}/calmodulin-dependant, it requires very low concentrations of Ca^{2+}/calmoduline for activity, and physiolgically relevant changes of intracellular Ca^{2+} are unlikely to cause a strong effect on eEF-2 kinase activity. On the other hand, it appears that changes in pH can drastically modulate eEF-2 kinase (see Preliminary Results). At pH$\geq$7.4, eEF-2 kinase activity is very low, and increased dramatically 10 to 20-fold upon a slight decrease in pH to 6.6. Thus, eEF-2 kinase appears to be a proton-activated protein kinase. It was also found that the pH-dependance of eEF-2 kinase activity closely correlated with pH dependance of protein synthesis inhibition. This observation suggests that eEF-2 kinase may be responsible for protein synthesis inhibition during acidification of the cytoplasm. Since significant acidification occurs during apoptosis, this acidification may result in the inhibition of protein synthesis due to activation of eEF-2 kinase and phosphorylation of eEF-2. Activation of eEF-2 kinase by acidic pH may explain the previously reported strong increase in eEF-2 phosphorylation seen in brain tissue from

Alzheimer's patients, as well as that seen in neurons after treatment with glutamate (35, 36).

The role of eEF-2 kinase in drug resistance: Activation of eEF-2 kinase by an acidic pH suggests that it may play an important role in regulation of protein synthesis during apoptosis. It is now well established that a significant decrease in intracellular pH is part of the apoptotic process. A drop in pH by 0.5-1 pH unit is universally observed during apoptosis induced by various agents, including anticancer drugs (37-43). It was found that at an intracellular pH of 6.5-6.8, eEF-2 becomes strongly phosphorylated. This phosphorylation of eEF-2 at an acidic pH may explain the inhibition of protein synthesis that was observed during apoptosis (44, 45).

Since apoptosis requires ongoing protein synthesis, phosphorylation of eEF-2, and the resulting inhibition of translation, may be an anti-apoptotic mechanism. Thus, it is suggested that eEF-2 phosphorylation is a cellular mechanism that can protect cells from apoptosis. To test this hypothesis, the effect of overexpression of eEF-2 kinase on drug resistance of mouse fibroblasts was tested. As is shown, overexpression of eEF-2 kinase increases approximately 10-fold the resistance of cells to different cytotoxic drugs, namely campotethicin and teniposide. This result suggests that eEF-2 kinase may be involved in modulation of drug resistance. Thus, inhibition of protein synthesis mediated by eEF-2 phosphorylation can protect cells from apoptotic cell death by various mechanisms that include up-and downregulation of expression of many genes. The pH-dependant increase in eEF-2 phosphorylation may also explain the previously observed dramatic increase in malignant cell resistance at low pH. It was demonstrated that at pH 6.5-6.8, different cell lines became more resistant to mitoxantrone, paclitaxel, and topotecan (46, 47).

Drug resistance related to activation of eEF-2 kinase can be particularly important in the hypoxic interior of solid tumors, which are characterized by acidic pH (48, 49). In fact, it was recently demonstrated that eEF-2 kinase activity is drastically

upregulated in tumor cell lines (161) and in invasive breast cancer specimens obtained from patients (162). Thus, eEF-2 kinase can be an important mechanism contributing to drug resistance of cancer cells.

Accordingly, specific inhibition of eEF-2 kinase can sensitize cancer cells to apoptitic cell death and to the cytotoxic action of anticancer drugs.

The results demonstrate that eEF-2 kinase was dramatically activated by a decease in pH within the range that occurs during apoptosis and that the pH-dependence of eEF-2 kinase activation correlated with protein synthesis inhibition *in vivo* and that overexpression of eEF-2 kinase in mouse fibroblasts increased their resistance to cytotoxic drugs. Using deletion mutagenesis, it was determined the tentative location of the various functional domains of eEF-2 kinase.

Activation of eEF-2 kinase by low pH: eEF-2 kinase is a Ca^{2+}/calmodulin-dependant enzyme whose only known substrate is eEF-2. Initially, it was suggested that the function of eEF-2 kinase was to phosphorylate eEF-2, and arrest protein synthesis in response to an elevation of Ca^{2+} levels in the cytoplasm. However, in a recent detailed study of the relationship between intracellular Ca^{2+} levels, eEF-2 phosphorylation, and protein synthesis in GH3 cells, it was shown that a superphysiological increase in Ca^{2+} levels in the cytoplasm produced only an insignificant increase in eEF-2 phosphorylation that did not affect translation (34).

Analysis of the pH dependence of recombinant GST-eEF-2 kinase demonstrated that both autophosphorylation activity and eEF-2 phosphorylation activity were markedly pH-dependent. The pH-dependence of eEF-2 kinase activity was quantitatively analyzed using a synthetic 16mer peptide substrate (MH-1; RKKFGESEKTKTKEFL-amide. MH-1 corresponds to the MHCK A phosphorylation site in *Dictyostelium* myosin heavy chains. It was found it to be an efficient substrate for eEF-2 kinase.

Activity of GST-eEF-2 kinase was assayed as follows: Purified GST-eEF-2 kinase was incubated with MH-1 (100 μM final) in a buffer consisting of 25 mM Hepes-KOH (pH varying), 5 mM magnesium acetate, 2.5 mM DTT, 50 μM $CaCl_2$, 0.5 μg calmodulin, 100 μM ATP, and 0.5 μCi [γ-^{33}P]-ATP (specific activity = 2000 Ci/mmol). The total volume of the reaction was 50 μl. The reaction was run at 30°C for various periods of time, and was terminated by incubation in an ice-water bath. An aliquot of each reaction was spotted onto a 2cm x 2 cm square of phosphocellulose paper and then washed 4 x 4 minutes in 75 mM phosphoric acid. After a 30 second rinse in 100% ethanol, the filter papers were dried, and then counted in a scintillation counter. To assay for autophosphorylation activity, kinase assays were run as above except that the peptide was omitted from the reaction mixture.

Figure 10 shows that phosphorylation of the peptide is very ineffective at pH $\geq$ 7.4, but when the pH drops to 6.6, eEF-2 kinase becomes dramatically activated. It was also analyzed how calmodulin activates eEF-2 kinase at different pH. eEF-2 kinase assays were done as described above, but with different concentrations of calmudulin at pH 6.6 and at pH 7.4. It appears that at pH 6.6, the K_a for calmoduline is about 10^9 M, while at pH 7.4, the K_a is approximately 10^{-7}. These results suggest that changes in intracellular Ca^{2+} at physiological pH-will not significantly affect eEF-2 kinase activity, which is consistent with recently reported results on GH3 cells (34).

To determine how specific this pH effect is for eEF-2 kinase, the pattern of protein phosphorylation at different pH in extracts from rat heart tissue was analyzed. A heart was dissected from a freshly killed rat and frozen in liquid nitrogen. The frozen tissue was homogenized in a buffer containing 25 mM Hepes-KOH (pH 7.4), 100 mM NaCl, 3mM EDTA, 2mM EGTA, 40 μg/ml soybean trypsin inhibitor, 0.5 mM PMSF, 20 mM Na pyrophophatase. The homogenate was clarified by centrifugation for 20 min. at 16,000 xg at 4°C. In order to assay for eEF-2 kinase activity, 10 μl of the homogenate was added to a reaction mixture containing 50 mM Hepes-KOH (at various pH), 10 mM magnesium acetate, 5 mM DTT, 100 μM $CaCl_2$, 0.5 μg calmodulin, 60

μM ATP, and 2 μCi [γ-^{33}P]-ATP (specific activity = 2000 Ci/mmol). The total volume of the reaction was 40μl. The reaction was run at 30°C for 5 minutes, and was terminated by incubation in an ice-water bath. Laemmli sample buffer was added, and the reaction mixture was boiled for 5 minutes. Samples were analyzed by 8% SDS-PAGE and autoradiography. As can be seen from Figure 3, eEF-2 was the only protein whose phosphorylation increased in response to a decrease in pH.

Next whether a decrease in pH_i of cells in culture resulted in inhibition of protein synthesis, and whether it correlated with activation of eEF-2 kinase were analyzed. Protein synthesis was measured in GH3 cells by [^{3}H]-Leu pulse-incorporation. GH3 cells were pre-incubated for 30 minutes in Ham's F-10 medium at 0.2 pH unit intervals from pH 6.0 to 8.0. Leucine pulse-incorporation was measured as described in Brostrom et al. (115). 1 mM Ca^{2+} and 100 μM [^{3}H]-Leu were added to the medium for a 15 minute incorporation period. Cells were harvested by centrifugation, washed, and lysed. Unincorporated label was removed by TCA precipitation, and Leu incorporation was measured by scintillation counting.

The actual intracellular pH was verified by incorporating BCECF/AM into GH3 cells in balanced salt solution buffered at pH 7.4 for 30 minutes. One set of cells was washed and re-suspended in normal balanced salt solutions at 0.2 pH unit intervals between 6.0 and 7.8. The other set of cells was re-suspended in high-K + buffer containing nigericin. A ratio of excitation of 485nm/440nm with emission at 530 nm was determined for each sample and pH_i was calculated as described by Thomas et al. (116).

The results of these experiments are shown in Figure 11. A decrease in the intracellular pH produced a strong inhibition of protein synthesis, which indeed correlated with the pH-dependence of eEF-2 kinase activity . These results demonstrate that eEF-2 phosphorylation may be a mechanism responsible for inhibiting protein synthesis at a low intracellular pH. Since the decrease in intracellular pH to the level

that strongly activates eEF-2 kinase is universally observed during apoptosis (37-43), this provides evidence that eEF-2 phosphorylation may be responsible for protein synthesis inhibition during apoptosis.

Effect of overexpression of eEF-2 kinase on the cellular resistance to anticancer drugs: Cell lines overexpressing eEF-2 kinase by stably transfecting N1H/3T3 cells with full length murine eEF-2 kinase cDNA were created. Murine eEF-2 kinase cDNA was cloned into pCMV-SPORT2 (GIBCO/BRL) under the control of a CMV promoter. N1H/3T3 cells were co-transfected with pCMV-SPORT2-EF2K and pSV2neo, using Lipofectamine (GIBCO/BRL). Stable transformants were selected with G_{418} (0.5 mg/ml), and were further maintained in medium containing G_{418}. A control cell line (C13) was produced by co-transfection of N1H/3T3 cells with empty pCMV-SPORT2/pSV2neo. The expression of eEF-2 kinase mRNA in the selectants was assayed by Northern blot analysis. Two cloned cell lines, designated as E8 and E9 were found to overexpress significantly eEF-2 kinase mRNA, with E9 giving the maximal level of expression.

In order to verify that overexpression of eEF-2 kinase mRNA in E8 and E9 produced an increase in eEF-2 kinase activity, cell lysates were assayed for the ability to phosphorylate eEF-2. eEF-2 kinase assays were performed as described above. E8 and E9 indeed showed greatly increased eEF-2 kinase activity in comparison to control cells. Overexpression of eEF-2 kinase did not have any effect on the growth parameters of cells. Cell cycle distribution of exponenentially growing or quiescent cells, as well as growth rates, were the same for clones 8, 9 and control clones.

To answer the question whether eEF-2 kinase plays a role in resistance, clone E9 overexpressing eEF-2 kinase was incubated with two cytotoxic drugs, and assessed cell survival by MTT cytotoxicity assay. E9 and C13 were plated in 96-well plates at 3000 cells per well. Cells were grown in DMEM with 10% FCS for 1 day, and then incubated with different concentrations of camptothecin (CPT) or teniposide (VM26)

for 4 days, and MTT assays were performed (Figure 12). E9 showed significantly increased resistance to these drugs.

EXAMPLE 11

Expression and mutagenesis of recombinant human eEF2 kinase.

Methods for efficient expression of recombinant eEF-2 kinase in bacteria, as well as generated and analyzed thirteen deletion mutants were developed. Expression of wild-type 6xHis-tagged and GST-tagged human eEF-2 kinase in *E. coli.* Initially, human eEF-2 kinase was expressed as a fusion protein with 6xHis or glutathione-S-transferase (GST). Human eEF-2 kinase cDNA was cloned into two different expression vectors: pRSET (Invitrogen). and pGEX-2T (Pharmacia). After the resulting vectors were transformed into *E. coli* strain JM109(DE3), the transformants were cultured in LB broth containing 50 mg/ml ampicillin. At log phase growth, isopropyl-b-thiogalactopyranoside (IPTG) was added to the bacterial cultures to a final concentration of 0.5 mM. After three hours, the cultures were harvested and the cells were sonicated.

Both 6xHis-tagged and GST-tagged eEF-2 kinase were efficiently expressed as judged by the appearance of one major band on Coomassie-stained gels after SDS-PAGE analysis of crude lysates. Therefore, eEF-2 kinase activity was analyzed directly in the crude lysates. In order to assay for eEF-2 kinase activity, bacterial lysates were incubated with purified rabbit reticulocyte eEF-2 (0.5 mg) in a buffer consisting of 50 mM Hepes-KOH (pH 7.4), 10 mM magnesium acetate, 5 mM DTT, 100 mM $CaCl_2$, 0.5 mg calmodulin, 60 mM ATP, and 2 mCi [g-^{33}P]-ATP (specific activity = 2000 Ci/mmol). The total volume of the reaction was 40 ml. The reaction was run at 30¡C for 10 minutes, and was terminated by incubation in an ice-water bath. Laemmli sample buffer was added, and the reaction mixture was boiled for 5 minutes. Samples were analyzed by 8% SDS-PAGE. The gel was dried and exposed to film overnight.

As can be seen in Figure 13, recombinant eEF-2 kinase expressed in *E. coli* undergoes autophosphorylation upon incubation with [g-^{33}P]-ATP and can efficiently phosphorylate eEF-2. The ability of eEF-2 kinase to undergo autophosphorylation was previously reported for the enzyme purified from rabbit reticulocyte lysate (139). Autophosphorylated eEF-2 kinase was represented by two or three distinct bands, showing that autophosphorylation of eEF-2 kinase occurs at multiple sites, and slightly affects its mobility in the gel. Both the ability of eEF-2 kinase to autophosphorylate and phosphorylate eEF-2 were strictly calmodulin-dependent (see Figure 13).

Localization of eEF-2 kinase functional domains by *in vitro* mutagenesis: Using *in vitro* mutagenesis. thirteen mutants of eEF-2 kinase were obtained with deletions ranging from 36 to 76 amino acids that systematically span the entire

eEF-2 kinase molecule: *In vitro* mutagenesis was done with the Muta-Gene Phagemid *In Vitro* Mutagenesis kit from Bio-Rad which is based on a method developed by Kunkel (117). Human eEF-2 kinase cDNA was cloned into pCR2.1 (Invitrogen). This plasmid has an f1 ori so that it can exist as single-stranded, as well as double-stranded DNA. It also carries the genes for ampicillin and kanamycin resistance. Thus, this plasmid was found to be suitable for use with the Muta-Gene kit.

The construct was transformed into *E. coli* strain CJ236, a *dut ung* strain. The enzymes dUTPase and uracil-N-glycosylase have been mutated and are non-functional, thus allowing for a large pool of uracil to be maintained in the cell as well as to allow uracil to be incorporated into the replicated plasmid. Bacteria carrying the plasmid were selected by growth in medium containing ampicillin. In order to produce single-stranded DNA, cultures of CJ236 were infected with helper phage M13K07. Replication of the M13K07 genome has been partly disabled and, thus, the low copy number of the M13K07 genome allows for more packaging of the plasmid instead of helper phage. This minimizes contamination of the harvested single-stranded DNA with M13K07 DNA. There is a kanamycin resistance marker in the M13K07 genome which allows for

selection of infected bacteria by growing them in the presence of kanamycin. After purification from the helper phage, the single-stranded DNA was mutagenized. 30-mer oligonucleotides were synthesized which were complementary to a stretch of fifteen nucleotides on each side of the region to be deleted. Annealing of the oligo to single-stranded DNA caused the region to be deleted to loop out. The oligo acted as a primer for second- strand synthesis by T7 DNA polymerase. Synthesis was done in the presence of thymidine so that the resulting double-stranded construct was a hybrid of a uracil-containing strand and a thymidine-containing strand. The mutant plasmid was then transformed into *E. coli* strain DH5a. This strain has a functional uracil-N-glycosylase which inactivates the uracil-containing strand and allows the thymidine-containing strand to be replicated. After purification, the mutant plasmid was sequenced to verify that the proper deletion was made. Initially, the resulting thirteen mutants were expressed in vitro in a coupled transcription/translation system and assayed for eEF-2 kinase activity.

Although using an *in vitro* transcription/translation system is fast and efficient, there are two disadvantages. First, it is not quantitative. Second, the amount of protein expressed *in vitro* is very low. To overcome this problem, the thirteen mutants were cloned into pGEX-2T (Pharmacia), and expressed as GST fusion proteins in *E. coli.*

Expression of GST-eEF-2 kinase deletion mutants in bacteria and purification. All thirteen mutants were expressed in *E. coli* BL21(DE3) as GST-tagged proteins. Expression of GST-tagged human eEF-2 kinase was done as follows: the cDNA for the mutant forms human eEF-2 kinase was cloned into pGEX-2T, and transformed into *E. coli* BL21(DE3). Cultures were grown at 37^{0}C, and at log phase growth, protein expression was induced with IPTG added to a final concentration of 0.5 mM. After a 3 hour incubation at 37^{0}C with IPTG, cells were harvested by centrifugation.

Since wild-type and mutant eEF-2 kinase were found to be highly insoluble, the proteins were purified from inclusion bodies. Bacterial cells were re-suspended in sonication buffer (phosphate-buffered saline, 1 mg/ml lysozyme, 3 mM EDTA, 40 mg/ml soybean

trypsin inhibitor, 0.5 mM PMSF, 20 mM Na pyrophosphate) and sonicated. Lysate was centrifuged at 16,000xg for 30 minutes at 4^{0}C to pellet inclusion bodies. Pellet was re-suspended in a buffer containing 20 mM Tris-HCl (pH 7.0), 100 mM NaCl, 7 mM b-mercaptoethanol, 6 M urea and incubated on ice for 20 minutes. Lysate was dialyzed overnight at 4^{0}C against buffer consisting of 20 mM Tris-HCl (pH 7.0). 100 mM NaCl, and 7 mM b-mercaptoethanol. Dialyzed material was centrifuged at 16,000 xg for 20 min. at 4^{0}C to remove any remaining insoluble material.

Glutathione-agarose (Sigma) was added to the dialysate (bed volume = 1/2000 of culture volume), and incubated at 4^{0}C for 2 hours with gentle shaking. Glutathione agarose was pelleted by centrifugation and washed 3x with PBS (10x bed volume). Bound GST-EF-2 kinase was eluted with 50 mM Tris-HCl (pH 8.0) containing 25 mM reduced glutathione (Volume of elution buffer = bed volume). Elution was done on ice for 20 minutes.

Analysis of activity of deletion mutants: The thirteen deletion mutants were assayed for the ability to phosphorylate eEF-2 and to undergo autophosphorylation. eEF-2 (Figure 14)kinase assays were performed as described above using purified rabbit reticulocyte eEF-2 as a substrate, and the pH of the reaction was 6.4. Mutants with deletions between amino acids 51-335 were neither able to phosphorylate eEF-2 nor to undergo autophosphorylation. On the other hand, deletions between amino acids 521-725 caused a loss of eEF-2 kinase activity, but not a loss of autophosphorylation activity. This demonstrates that the catalytic domain is located between amino acids 51-335 while the region between amino acids 521-725 is important for eEF-2 recognition. The region between amino acids 336-520 probably serves as a hinge between two domains.

A summary of the results of mutational analysis of human eEF-2 kinase is shown in Figure 14. Mutational analysis reveals that eEF-2 kinase can be subdivided into two domains connected by a hinge region. The N-terminal domain represents the catalytic domain, and the C-terminal domain represents the eEF-2 targeting domain. This is consistent with the location of the catalytic domain as predicted by comparison of eEF-2

kinases from different species (27). As was discussed in the Background and Significance section, the amino acid sequence of this region displays no homology to the catalytic domains of the conventional protein kinases, but appears to be highly similar to the catalytic domains of the recently described myosin heavy chain kinases from *Dictyostelium*. Further experiments will define more precisely the location of the functional domains.

The results clearly demonstrate that the location of the catalytic domain of rat eEF-2 kinase suggested by Redpath *et al.* (140) in between amino acids 288-554 is incorrect.

Location of calmodulin-binding domain: In all mutants able to undergo autophosphorylation. this phosphorylation was strictly calcium/ calmodulin-dependent, suggesting that the calmodulin-binding region is located within amino acids 51 to 335. To locate the calmodulin-binding domain more precisely, five mutants were analyzed with deletions between amino acids 51 to 355 for their ability to bind calmodulin-agarose. The only mutant of eEF-2 kinase not able to bind calmodulin-agarose contains a deletion of amino acids 51-96. The actual calmodulin-binding site is probably within amino acids 81 to 94 (FKEAWKHAIQKAKH). which are predicted to form an amphipathic α-helix. The homologous sequence from *C. elegans* eEF-2 kinase (LMETWRKAARRART) is also predicted to form an amphipathic α-helix.

Expression of correctly-folded eEF-2 kinase: Although GST- and 6xHis-eEF-2 kinase expressed in bacteria was enzymatically active, the majority of recombinant protein ended up in inclusion bodies. The inclusion bodies were precipitated, dissolved in 8M urea, and dialyzed overnight against 20 mM Tris buffer (pH 7.0) containing 100 mM NaCl and 4 mM b-mercaptoethanol. The refolded protein was analyzed by SDS-PAGE and assayed for the ability to undergo autophosphorylation and to phosphorylate eEF-2. Preparations of refolded GST-eEF-2 kinase and 6xHis-eEF-2 kinase contained predominantly one band corresponding to eEF-2 kinase. In the case of GST-tagged eEF-

2 kinase, the protein was further purified using glutathione-Sepharose. Gel filtration analysis of recombinant eEF-2 kinase on Superdex-200 revealed that both 6xHis- and GST-eEF-2 kinase eluted predominantly in the void volume, indicating that it was aggregated. This aggregated kinase was enzymatically inactive. eEF-2 kinase activity eluted in the fractions corresponding to a Stokes radius of 52. Approximately 4% of the refolded eEF-2 kinase eluted in this area. The specific activity of eEF-2 kinase was 5 mmoles/min/mg, which is slightly higher than the specific activity of eEF-2 kinase purified from rabbit reticulocytes. The extremely low yield of active eEF-2 kinase prompted to search for alternative methods for expression of recombinant kinase.

eEF-2 kinase was expressed as fusion with thioredoxin in order to obtain a soluble protein because it was reported that thioredoxin can prevent accumulation of recombinant proteins in inclusion bodies (149). Human eEF-2 kinase cDNA was cloned into the expression vector pThioHis (Invitrogen). The thioredoxin expressed from this vector has additional histidine residues so it can also function as a His tag. Tthioredoxin-eEF-2 kinase was found to remain soluble and was enzymatically active. Moreover, its elution profile on Mono Q and Superdex-200 was very similar to eEF-2 kinase purified from rabbit reticulocytes, which shows that it was correctly folded. This His-thioredoxin-tagged kinase can be efficiently and easily purified from total lysates by immobilized metal affinity chromatography (IMAC) or by ThioBond resin (Invitrogen). This fusion protein also contains an enterokinase cleavage site, which allows for removal of the His-thioredoxin tag. Thus, a method to express active and, most likely correctly folded, eEF-2 kinase which will facilitate the studies was demonstrated.

Phosphorylation of synthetic peptides by eEF-2 kinase: Redpath *et al.* (118) were able to detect some [g-^{32}P]-ATP incorporation into a synthetic peptide corresponding to residues 49-60 of eEF-2 (RAGETRFTDTRK), which encompasses the phosphorylation site of eEF-2. The rate of phosphorylation was low, and the K_m was very high (>>2.5 mM). A larger peptide corresponding to amino acids 48-66 of eEF-2

(ARAGETRFTDTRKDEQERC), was synthesized and was unable to detect any phosphorylation after incubation with [g -^{33}P]-ATP and recombinant eEF-2 kinase.

As can be seen in Figure 15, eEF-2 kinase can efficiently phosphorylate MH-1. The K_m of this reaction was approximately 150 mM, which is similar to the K_m of the phosphorylation of MH-1 by MHCK A (105 mM; 119). If an a-helical conformation is necessary for recognition of MH-1 by eEF-2 kinase, then removal of the C-terminal amide from MH-1 should reduce the a-helical propensity of the peptide, and thus, make it a less efficient substrate for eEF-2 kinase. As can be seen in Figure 11a, this is the case. An uncapped version of the peptide (called MH-U) can serve as a substrate for eEF-2 kinase, although a significantly less efficient one. The reduction of phosphorylation efficiency is due entirely to a change in K_m, which for MH-U is approximately 400 mM.

It is interesting that the sequence of MH-1 is quite different from the sequence surrounding the phosphorylation site in eEF-2 (see Figure 15B). When looking at the primary structure, the threonines that undergo phosphorylation are surrounded in these two peptides by very different amino acids. But when the same sequences are folded into a-helices, the surrounding environment of those phosphoacceptor threonines is very similar, as can be seen in Figure 15B: in both cases, there is a basic amino acid to the left and a glutamate followed by a basic amino acid on the right. This pattern may represent the consensus sequence for recognition by eEF-2 kinase and the related protein kinases. Thus, as demonstrated herein eEF-2 kinase phosphorylates amino acids located within a-helices.

While the invention has been described and illustrated herein by references to various specific material, procedures and examples, it is understood that the invention is not restricted to the particular material combinations of material, and procedures selected for that purpose. Numerous variations of such details can be implied as will be appreciated by those skilled in the art.

WHAT IS CLAIMED IS:

1. A protein kinase which is a member of a superfamily, said protein kinase being characterized by:

A. greater than 40% sequence similarity with eEF-2 kinase from any organism; and,

B. phosphorylates an amino acid within an alpha helical domain of its target protein.

2. A protein kinase of Claim 1 which phosphorylates eukaryotic elongation factor-2 (eEF-2), and is designated as eukaryotic elongation factor-2 kinase (eEF-2 kinase).

3. A protein kinase of Claim 1 which phosphorylates eukaryotic myosin heavy chain (MHC), and is designated as myosin heavy chain kinase (MHCK).

4. A protein kinase of Claim 1 that phosphorylates a peptide sequence derived from the phosphorylation site of a target protein.

5. A peptide sequence having SEQ ID NO: 20.

6. A protein kinase of Claim 1 which is a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 10, and fragments thereof.

7. A protein kinase of Claim 1 which is derived from mammalian cells.

8. A protein kinase of Claim 1 labeled with a detectable label.

9. A protein kinase of Claim 8 wherein the label is selected from enzymes, chemicals which fluoresce, and radioactive elements.

10. An antibody to the protein kinase of Claim 1.

11. An antibody to the phosphorylated form of the target protein of Claim 1.

12. An antibody to the phosphorylated form of the peptide of Claim 5.

13. The antibody of Claim 10, 11 or 12 which is a polyclonal antibody.

14. The antibody of Claim 10, 11 or 12 which is a monoclonal antibody.

15. An immortal cell line that produces a monoclonal antibody according to Claim 14.

16. The antibody of Claim 10, 11 or 12 labeled with a detectable label.

17. The antibody of Claim 10, 11 or 12 wherein the label is selected from enzymes, chemicals which fluoresce and radioactive elements.

18. A DNA sequence which encodes eEF-2 kinase, or a fragment thereof, selected from the group consisting of:

(A) the DNA sequences of Figure 5 (SEQ ID NO: 1);

(B) the DNA sequences of Figure 5 (SEQ ID NO: 3);

(C) the DNA sequences of Figure 5 (SEQ ID NO: 9);

(D) DNA sequences that hybridize to any of the foregoing DNA sequences under standard hybridization conditions;

(E) DNA sequences that code for expression of an amino acid sequence encoded by any of the foregoing DNA sequences.

(F) degenerate variants thereof;

(G) alleles thereof; and,

(H) hybridizable fragments thereof.

19. A recombinant DNA molecule comprising a DNA sequence which encodes eEF-2 kinase, or a fragment thereof, selected from the group consisting of:

(A) the DNA sequences of Figure 5 (SEQ ID NO: 1);

(B) the DNA sequences of Figure 5 (SEQ ID NO: 3);

(C) the DNA sequences of Figure 5 (SEQ ID NO: 9);

(D) DNA sequences that hybridize to any of the foregoing DNA sequences under standard hybridization conditions;

(E) DNA sequences that code for expression of an amino acid sequence encoded by any of the foregoing DNA sequences.

(F) degenerate variants thereof;

(G) alleles thereof; and,

(H) hybridizable fragments thereof.

20. The recombinant DNA molecule of either of Claims 18 or 19, wherein said DNA sequence is operatively linked to an expression control sequence.

21. The recombinant DNA molecule of Claim 20, wherein said expression control sequence is selected from the group consisting of the early or late promoters of SV40 or adenovirus, the *lac* system, the *trp* system, the *TAC* system, the *TRC* system, the major operator and promoter regions of phage λ, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase and the promoters of the yeast α-mating factors.

22. A probe capable of screening for eEF-2 kinase in alternate species prepared from the DNA sequence of Claim 18.

23. A probe capable of screening for members of the protein kinase superfamily of Claim 1 prepared from the DNA sequence of Claim 18.

24. A unicellular host transformed with a recombinant DNA molecule comprising a DNA sequence or degenerate variant thereof, which encodes a protein kinase, or a fragment thereof, selected from the group consisting of:

(A) the DNA sequences of Figure 5 (SEQ ID NO: 1);

(B) the DNA sequences of Figure 5 (SEQ ID NO: 3);

(C) the DNA sequences of Figure 5 (SEQ ID NO: 9);

(D) DNA sequences that hybridize to any of the foregoing DNA sequences under standard hybridization conditions; and

(E) DNA sequences that code on expression for an amino acid sequence encoded by any of the foregoing DNA sequences;

wherein said DNA sequence is operatively linked to an expression control sequence.

25. The unicellular host of Claim 24 wherein the unicellular host is selected from the group consisting of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, yeasts, CHO, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, and BMT10 cells, plant cells, insect cells, and human cells in tissue culture.

26. A method for detecting eEF-2 kinase and assessing eEF-2 kinase levels by:

A. contacting a biological sample from a mammal in which the presence or activity of said eEF-2 kinase is suspected with a binding partner of said eEF-2 kinase under conditions that allow binding of said eEF-2 kinase to said binding partner to occur; and,

B. detecting whether binding has occurred, and to what degree, between said eEF-2 kinase from said sample and the binding partner;

wherein the detection of binding indicates that presence or activity of said eEF-2 kinase in said sample, and indicates a level of said eEF-2 kinase in the sample.

27. An assay system for screening drugs and other agents for ability to modulate eEF-2 kinase activity, comprising a predetermined amount of eEF-2 kinase mixed with varying amounts of drug or agent, along with target protein and ATP; wherein detection is *via* either a detectable label on the γ-phosphate of ATP, or on an antibody directed against the phosphorylated target protein..

28. The assay system of Claim 27 wherein the label on the γ-phosphate of ATP comprises one of the following:

A. ^{32}P;

B. ^{33}P

C. ^{35}S

D. a biotinylated phosphate moiety; or,

E. a fluorescent phosphate moiety.

29. The assay system of Claim 27 wherein the label on the antibody comprises one of the following:

A. an enzyme detectable with colorimetric, fluorescent, or chemiluminescent substrates, such as alkaline phosphatase or horseradish peroxidase;

B. a biotin moiety;

C. a fluorescent moiety; or,

D. a radioactive moiety chosen from the following group of isotopes: ^{3}H, ^{14}C, ^{32}P, ^{33}P. ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{90}Y, ^{125}I, ^{131}I, and ^{186}Re.

30. An assay system for screening drugs and other agents for ability to modulate eEF-2 kinase activity, comprising:

A. culturing an observable cellular test colony inoculated with a drug or agent;

B. harvesting a supernatant from said cellular test colony; and,

C. examining said supernatant for the presence of said eEF-2 kinase activity wherein an increase or a decrease in a level of said eEF-2 kinase activity indicates the ability of a drug to modulate the activity of said eEF-2 kinase.

31. A test kit for assessing the level of eEF-2 kinase activity in a eukaryotic cellular sample, comprising:

A. a predetermined amount of a detectably labelled specific binding partner of eEF-2 kinase.

B. other reagents; and,

C. directions for use of said kit.

31. The test kit of Claim 31 wherein said labeled immunochemically reactive component is selected from the group consisting of polyclonal antibodies to eEF-2 kinase, monoclonal antibodies to eEF-2 kinase, fragments thereof, and mixtures thereof.

32. A method of preventing and/or treating cellular debilitations, derangements and/or dysfunctions and/or other disease states in mammals, comprising administering to a mammal a therapeutically effective amount of a material selected from the following group:

A. peptides that inhibit eEF-2 kinase;

B. antibodies against eEF-2 kinase; and,

C. other drugs or agents that specifically inhibit eEF-2 kinase.

33. A pharmaceutical composition for the treatment of cellular debilitation, derangement and/or dysfunction in mammals, comprising:

A. a therapeutically effective amount of a material selected from the group consisting of: peptides that inhibit eEF-2 kinase; antibodies against eEF-2 kinase; and, other drugs or agents that specifically inhibit eEF-2 kinase; and,

B. a pharmaceutically acceptable carrier.

34. A recombinant virus transformed with the DNA molecule, or a derivative or fragment thereof, in accordance with Claim 18.

35. A recombinant virus transformed with the DNA molecule, or a derivative or fragment thereof, in accordance with Claim 19.

36. The recombinant DNA molecule of Claim 20 comprising plasmid pGEX-3X, clone E3 or plasmid pGEX-3X, clone E4.

37. An antisense nucleic acid against eEF-2 kinase mRNA comprising a nucleic acid sequence hybridizing to said mRNA.

38. The antisense nucleic acid of Claim 37 which is RNA.

39. The antisense nucleic acid of Claim 37 which is DNA.

40. The antisense nucleic acid of Claim 37 which binds to the initiation codon of any of said mRNAs.

41. A recombinant DNA molecule having a DNA sequence which, on transcription, produces an antisense ribonucleic acid against eEF-2 kinase mRNA, said antisense ribonucleic acid comprising an nucleic acid sequence capable of hybridizing to said mRNA.

42. A eEF-2 kinase-producing cell line transfected with the recombinant DNA molecule of Claim 41.

43. A method for creating a cell line which exhibits reduced expression of eEF-kinase, comprising transfecting a eEF-2 kinase-producing cell line with a recombinant DNA molecule of claim 41.

44. A ribozyme that cleaves eEF-2 kinase mRNA.

45. The ribozyme of Claim 44 which is a *Tetrahymena*-type ribozyme.

46. The ribozyme of Claim 44 which is a Hammerhead-type ribozyme.

47. A recombinant DNA molecule having a DNA sequence which, upon transcription, produces the ribozyme of claim 44.

48. A eEF-2 kinase-producing cell line transfected with the recombinant DNA molecule of claim 47.

49. A method for creating a cell line which exhibits reduced expression of eEF-2 kinase, comprising transfecting a eEF-2 kinase-producing cell line with the recombinant DNA molecule of claim 44.

50. An isolated nucleic acid encoding a protein kinase which is a member of a superfamily, said protein kinase being characterized by: A. greater than 40% sequence similarity with eEF-2 kinase from any organism; and,B.phosphorylates an amino acid within an alpha helical domain of its target protein.

51. The isolated nucleic acid of claim 50, wherein the nucleic acid encodes eEF-2 kinase protein, heart protein kinase, melanoma protein protein, or ch4 protien kinase.

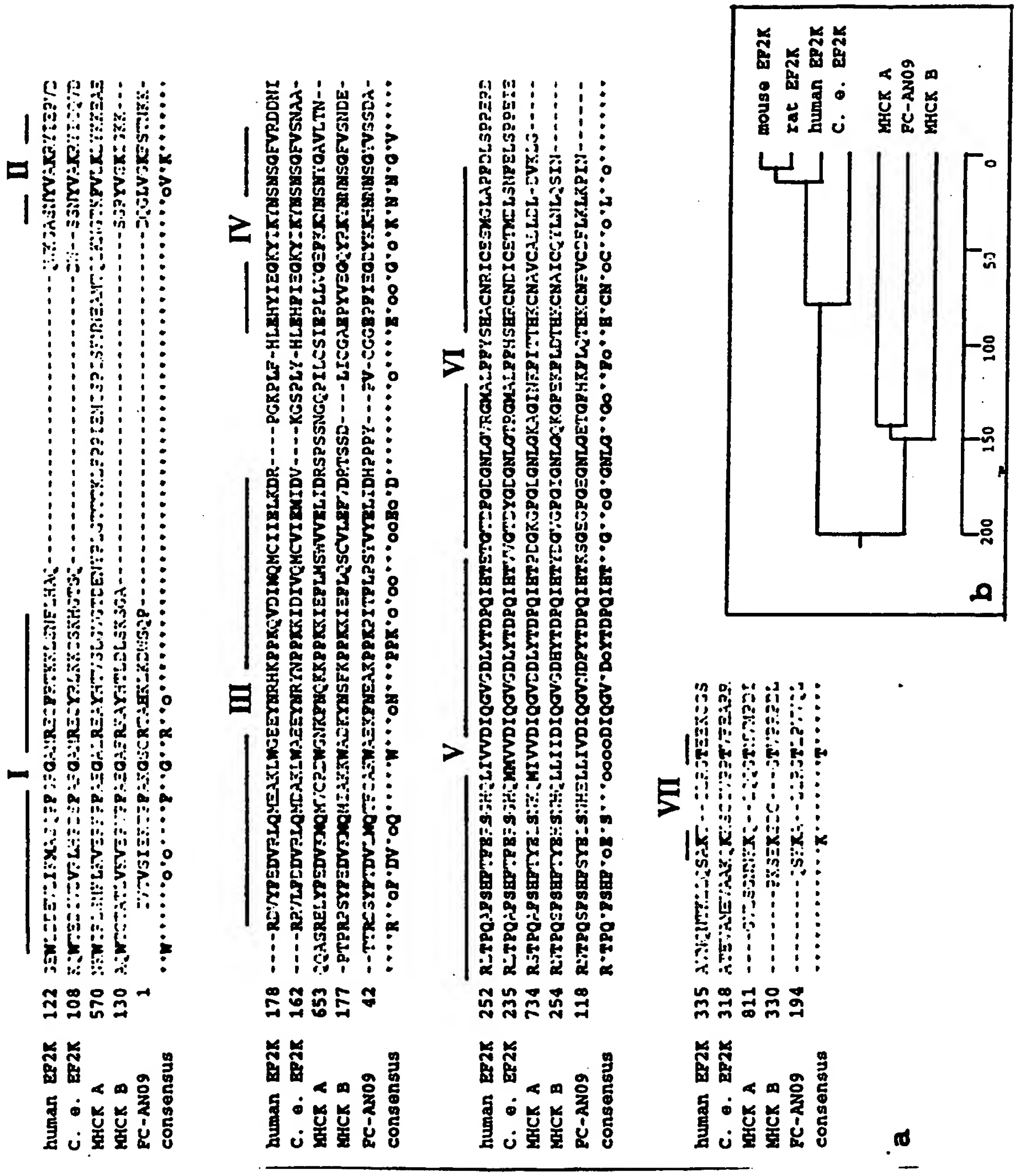

I
II
III
IV
V
VI
VII
human EP2K
C. e. EP2K
MHCK A
MHCK B
PC-AN09
consensus
a
mouse EP2K
rat EP2K
human EP2K
C. e. EP2K
MHCK A
PC-AN09
MHCK B
0
50
100
150
200
b

FIGURE 1

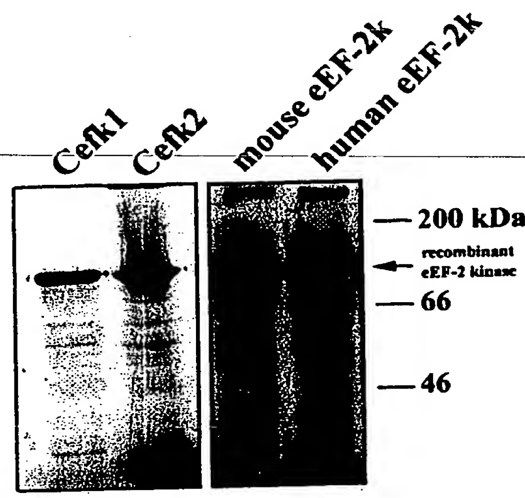

Cefk1
Cefk2
mouse eEF-2k
human eEF-2k
200 kDa
recombinant eEF-2 kinase
66
46

FIGURE 2

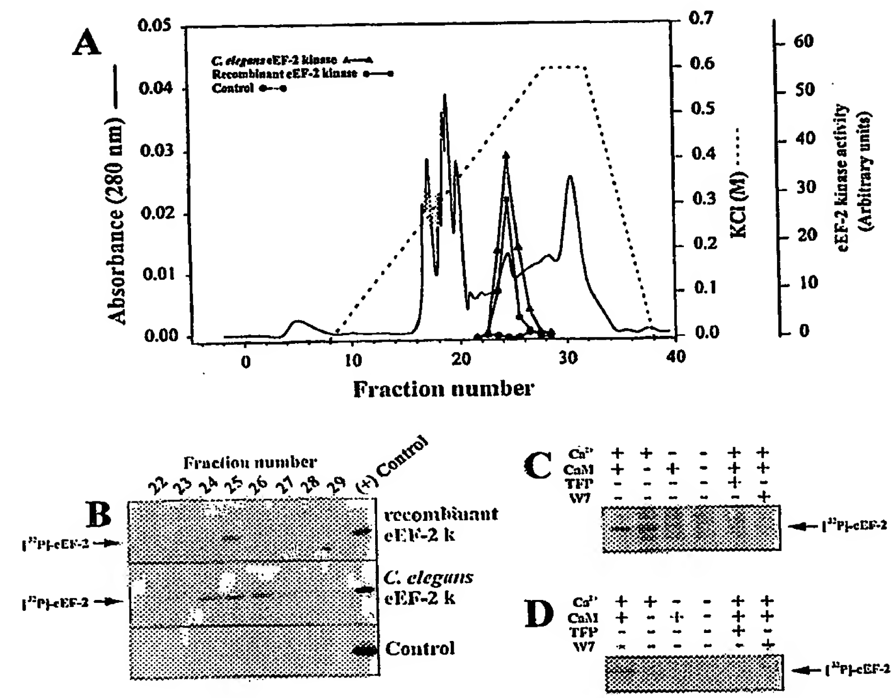

A
C. elegans eEF-2 kinase
Recombinant eEF-2 kinase
Control
Absorbance (280 nm)
KCl (M)
eEF-2 kinase activity (Arbitrary units)
Fraction number
B
Fraction number
22 23 24 25 26 27 28 29 (+) Control
recombinant eEF-2 k
C. elegans eEF-2 k
Control
[32P]-eEF-2
C
D
Ca2+
CaM
TFP
W7

FIGURE 3

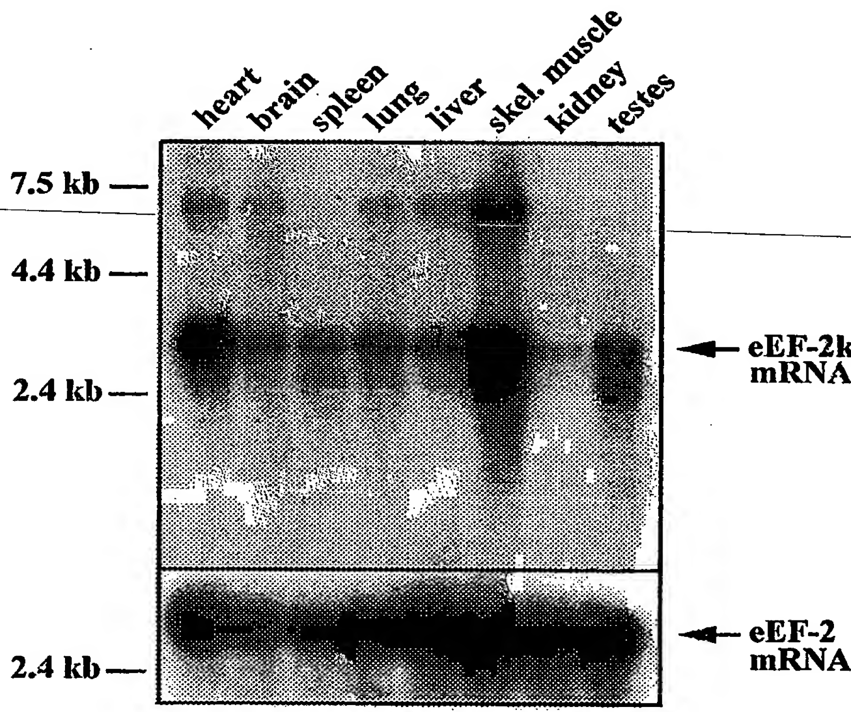

heart
brain
spleen
lung
liver
skel. muscle
kidney
testes
7.5 kb
4.4 kb
2.4 kb
eEF-2k mRNA
2.4 kb
eEF-2 mRNA

FIGURE 4

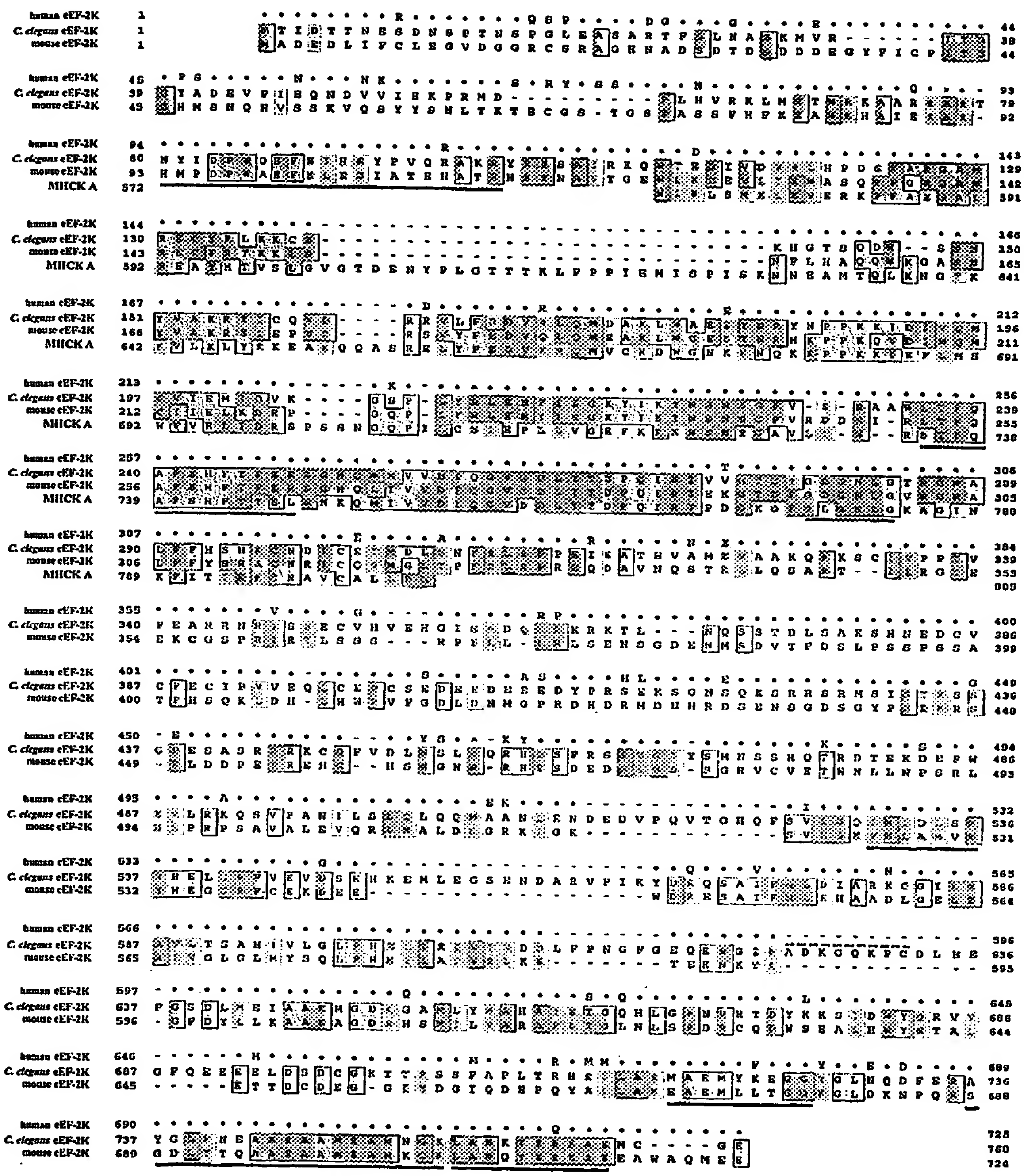


FIGURE 5

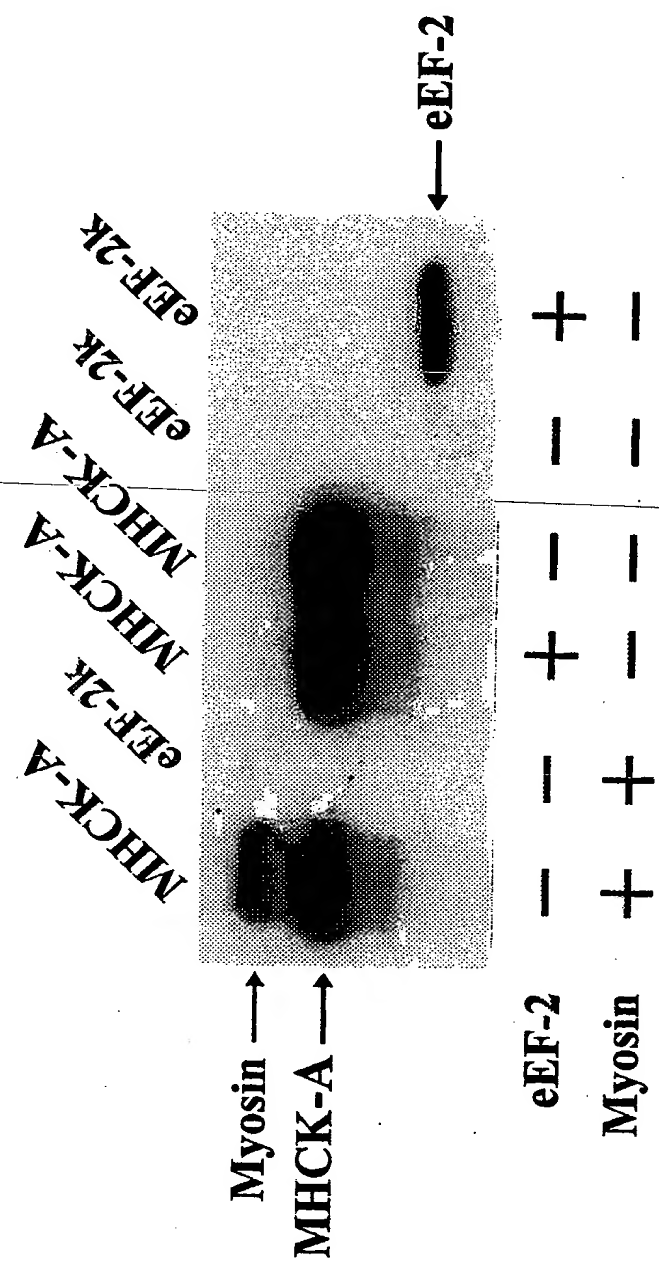

eEF-2
eEF-2k
eEF-2k
MHCK-A
MHCK-A
eEF-2k
MHCK-A
Myosin
MHCK-A
eEF-2
Myosin

FIGURE 6

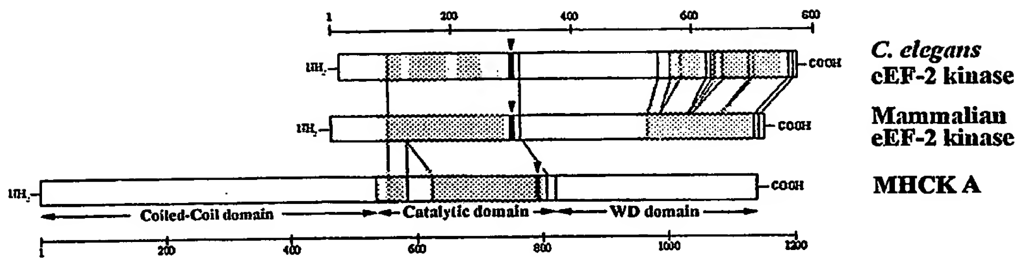
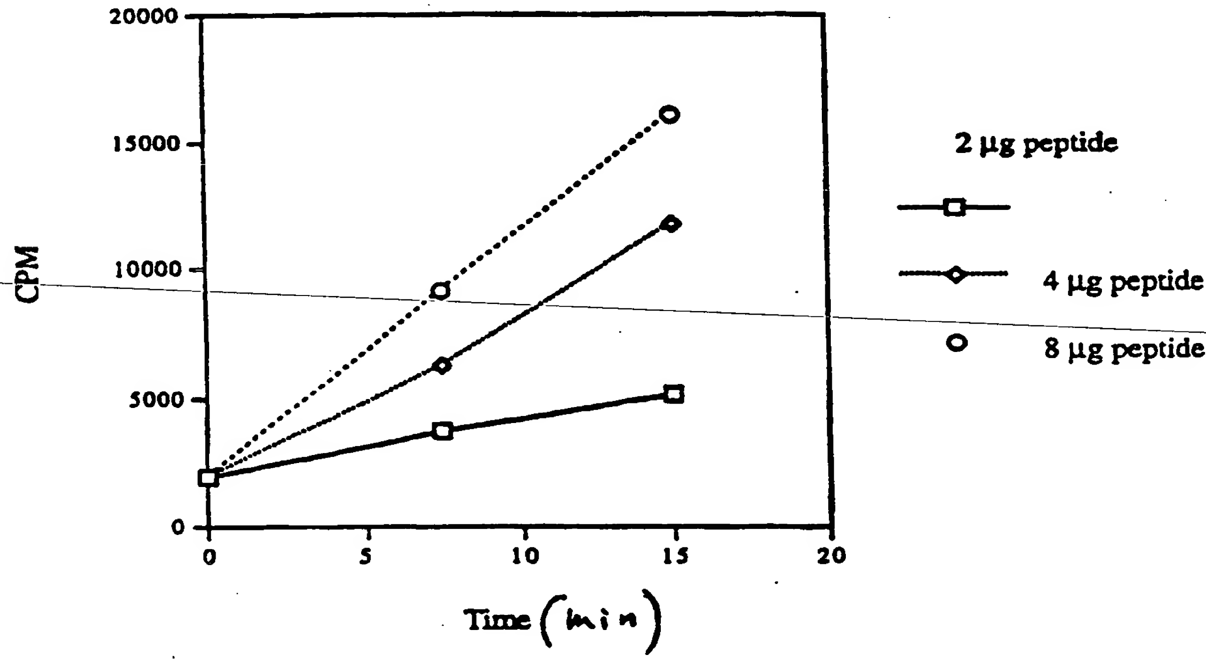

1
200
400
600
800
NH2
COOH
C. elegans
cEF-2 kinase
Mammalian
eEF-2 kinase
MHCK A
Coiled-Coil domain
Catalytic domain
WD domain
1
200
400
600
800
1000
1200

FIGURE 7


20000
15000
10000
5000
0
CPM
0
5
10
15
20
Time (min)
2 µg peptide
4 µg peptide
8 µg peptide

FIUGRE 8

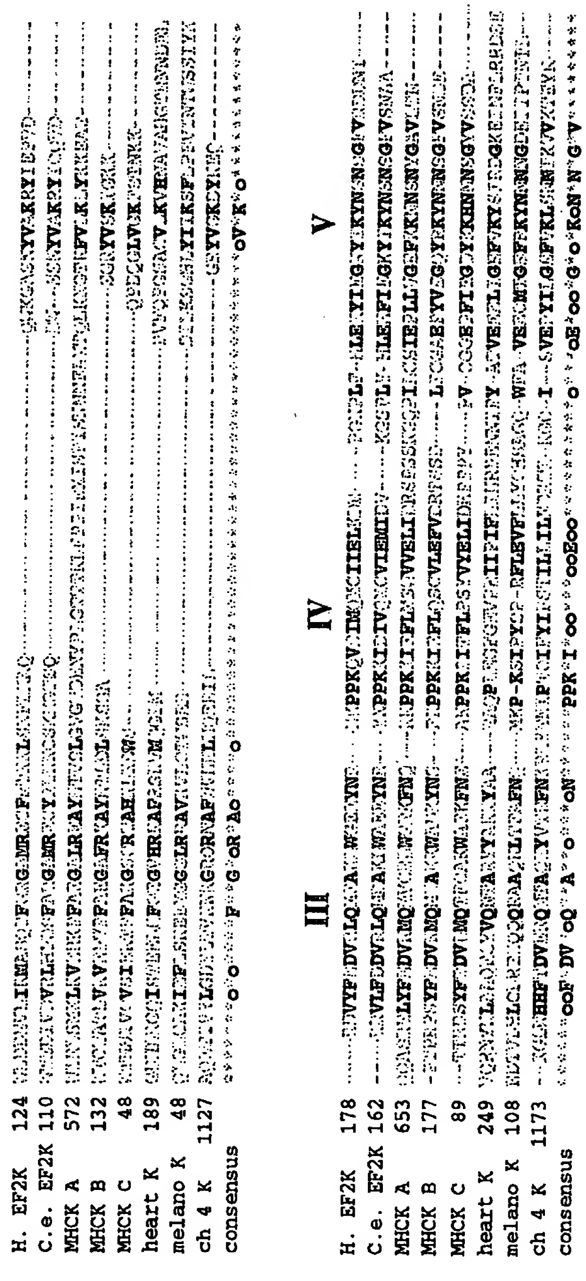


FIGURE 9

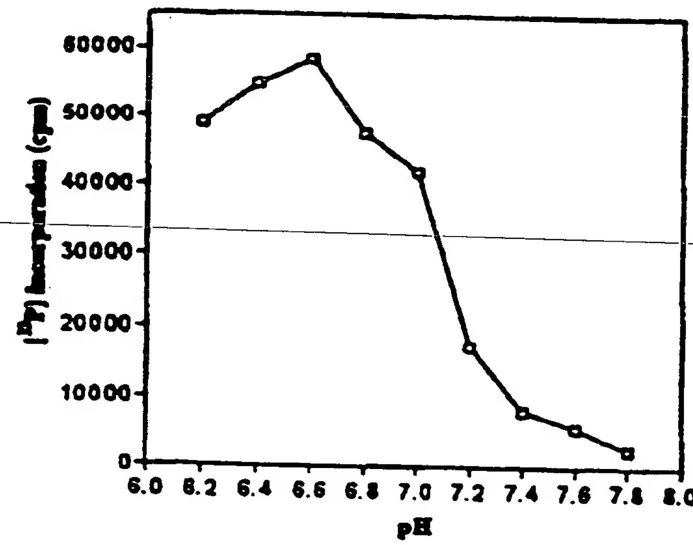

60000
50000
40000
30000
20000
10000
0
6.0
6.2
6.4
6.6
6.8
7.0
7.2
7.4
7.6
7.8
8.0
pH

FIGURE 10

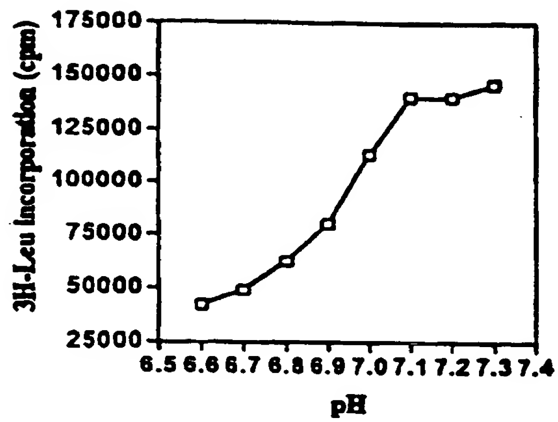

3H-Leu Incorporation (cpm)
175000
150000
125000
100000
75000
50000
25000
6.5
6.6
6.7
6.8
6.9
7.0
7.1
7.2
7.3
7.4
pH

FIGURE 11

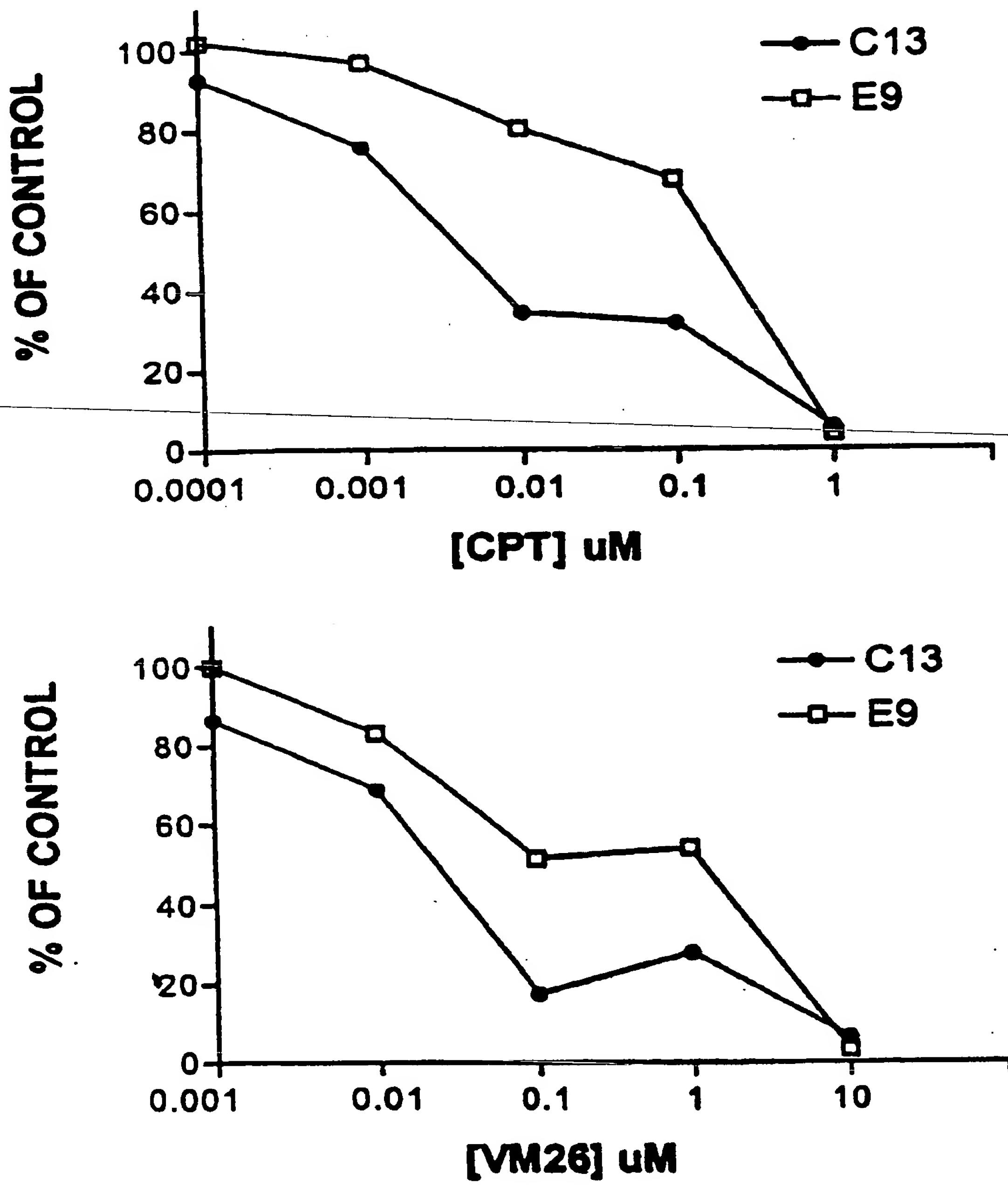

% OF CONTROL
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
[CPT] uM
C13
E9
% OF CONTROL
100
80
60
40
20
0
0.001
0.01
0.1
1
10
[VM26] uM
C13
E9

FIGURE 12

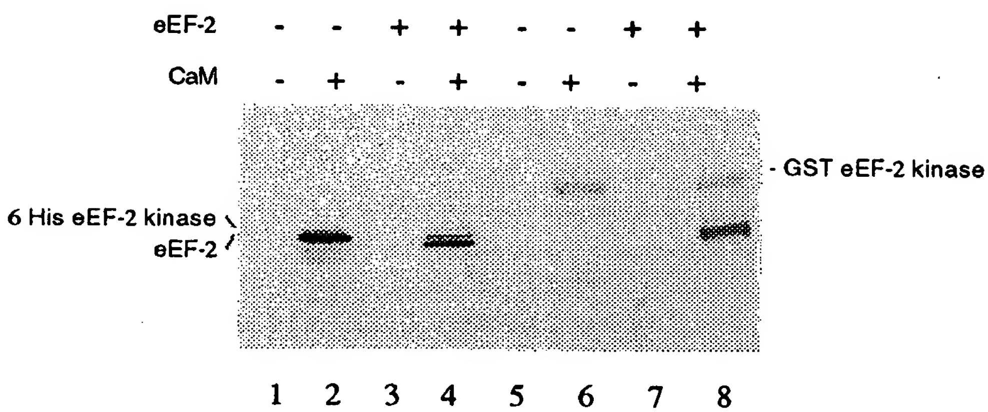


FIGURE 13

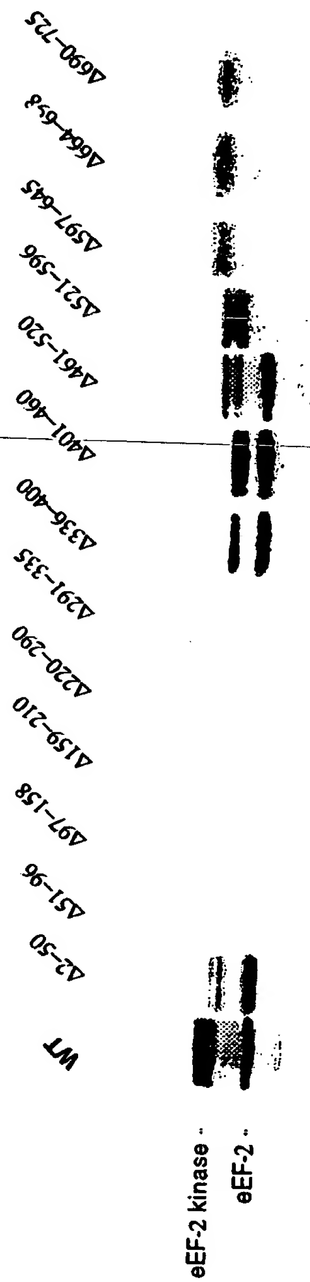

Δ690-725
Δ597-645
Δ521-596
Δ461-520
Δ401-460
Δ336-400
Δ291-335
Δ220-290
Δ159-210
Δ97-158
Δ51-96
Δ2-50
WT
eEF-2 kinase
eEF-2

FIGURE 14

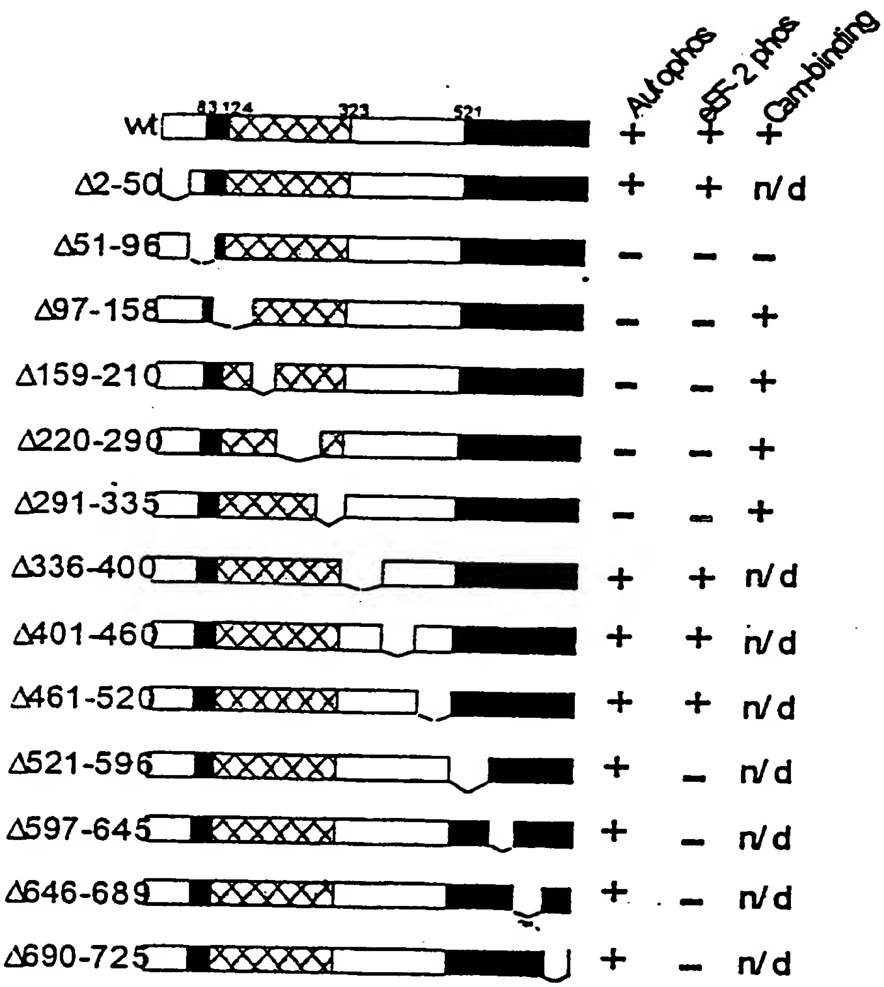
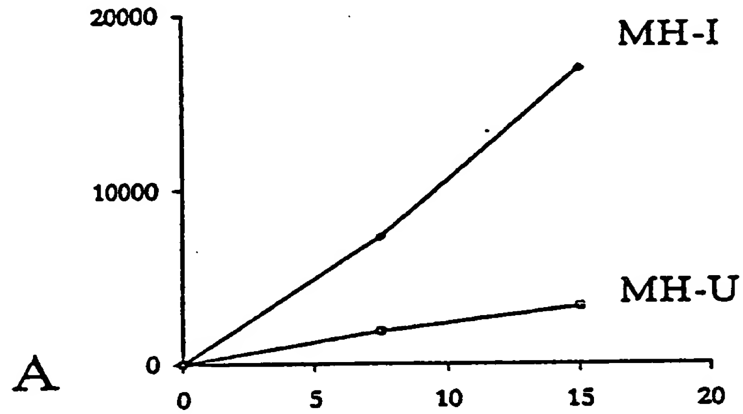

Autophos
eEF-2 phos
Cam-binding
83
124
323
521
wt + + +
Δ2-50 + + n/d
Δ51-96 − − −
Δ97-158 − − +
Δ159-210 − − +
Δ220-290 − − +
Δ291-335 − − +
Δ336-400 + + n/d
Δ401-460 + + n/d
Δ461-520 + + n/d
Δ521-596 + − n/d
Δ597-645 + − n/d
Δ646-689 + − n/d
Δ690-725 + − n/d

FIGURE 15



*
RKKFGESEKTKTKEFL - MHC
SARAGETRFTDTRKDE - EF2

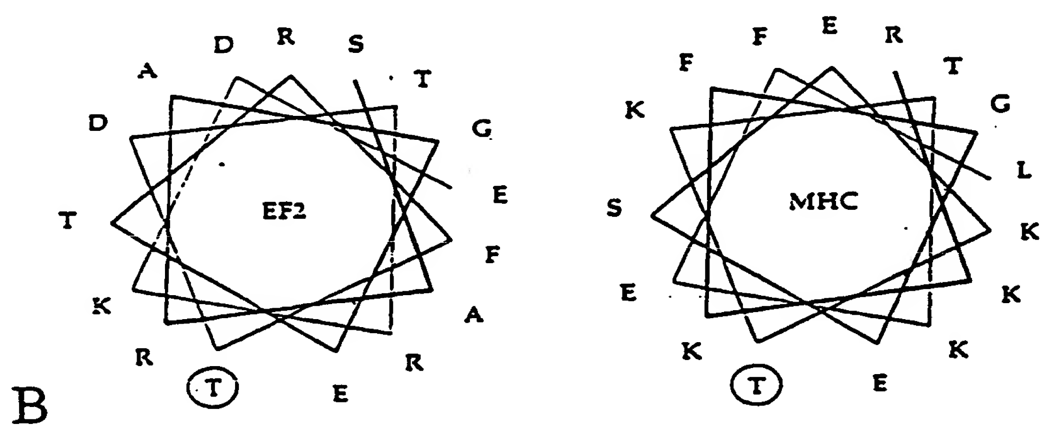


FIGURE 16

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Ryazanov, Alexey G.
Hait, William N.
Pavur, Karen S.

(ii) TITLE OF INVENTION: ELONGATION FACTOR-2 KINASE (EF-2 KINASE) AND METHODS OF USE THEREFOR

(iii) NUMBER OF SEQUENCES: 25

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: David A. Jackson, Esq.
(B) STREET: 411 Hackensack Ave, Continental Plaza, 4th Floor
(C) CITY: Hackensack
(D) STATE: New Jersey
(E) COUNTRY: USA
(F) ZIP: 07601

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Jackson Esq., David A.
(B) REGISTRATION NUMBER: 26,742
(C) REFERENCE/DOCKET NUMBER: 601-1-078

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 201-487-5800
(B) TELEFAX: 201-343-1684

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2178 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ATGGCAGACG AAGACCTCAT CTTCCGCCTG GAAGGTGTTG ATGGCGGCCA GTCCCCCCGA      60
GCTGGCCATG ATGGTGATTC TGATGGGGAC AGCGACGATG AGGAAGGTTA CTTCATCTGC     120
CCCATCACGG ATGACCCAAG CTCGAACCAG AATGTCAATT CCAAGGTTAA TAAGTACTAC     180
AGCAACCTAA CAAAAAGTGA GCGGTATAGC TCCAGCGGGT CCCCGGCAAA CTCCTTCCAC     240
TTCAAGGAAG CCTGGAAGCA CGCAATCCAG AAGGCCAAGC ACATGCCCGA CCCCTGGGCT     300
GAGTTCCACC TGGAAGATAT TGCCACCGAA CGTGCTACTC GACACAGGTA CAACGCCGTC     360
ACCGGGGAAT GGCTGGATGA TGAAGTTCTG ATCAAGATGG CATCTCAGCC CTTCGGCCGA     420
GGAGCAATGA GGGAGTGCTT CCGGACGAAG AAGCTCTCCA ACTTCTTGCA TGCCCAGCAG     480
TGGAAGGGCG CCTCCAACTA CGTGGCGAAG CGCTACATCG AGCCCGTAGA CCGGGATGTG     540
TACTTTGAGG ACGTGCGTCT ACAGATGGAG GCCAAGCTCT GGGGGGAGGA GTATAATCGG     600
CACAAGCCCC CCAAGCAGGT GGACATCATG CAGATGTGCA TCATCGAGCT GAAGGACAGA     660
CCGGGCAAGC CCCTCTTCCA CCTGGAGCAC TACATCGAGG GCAAGTACAT CAAGTACAAC     720
TCCAACTCTG GCTTTGTCCG TGATGACAAC ATCCGACTGA CGCCGCAGGC CTTCAGCCAC     780
TTCACTTTTG AGCGTTCCGG CCATCAGCTG ATAGTGGTGG ACATCCAGGG AGTTGGGGAT     840
CTCTACACTG ACCCACAGAT CCACACGGAG ACGGGCACTG ACTTTGGAGA CGGCAACCTA     900
GGTGTCCGCG GGATGGCGCT CTTCTTCTAC TCTCATGCCT GCAACCGGAT TTGCGAGAGC     960
ATGGGCCTTG CTCCCTTTGA CCTCTCGCCC CGGGAGAGGG ATGCAGTGAA TCAGAACACC    1020
AAGCTGCTGC AATCAGCCAA GACCATCTTG AGAGGAACAG AGGAAAAATG TGGGAGCCCC    1080
CGAGTAAGGA CCCTCTCTGG GAGCCGGCCA CCCCTGCTCC GTCCCCTTTC AGAGAACTCT    1140
GGAGACGAGA ACATGAGCGA CGTGACCTTC GACTCTCTCC CTTCTTCCCC ATCTTCGGCC    1200
```

```
ACACCACACA GCCAGAAGCT AGACCACCTC CATTGGCCAG TGTTCAGTGA CCTCGATAAC    1260
ATGGCATCCA GAGACCATGA TCATCTAGAC AACCACCGGG AGTCTGAGAA TAGTGGGGAC    1320
AGCGGATACC CCAGTGAGAA GCGGGGTGAG CTGGATGACC CTGAGCCCCG AGAACATGGC    1380
CACTCATACA GTAATCGGAA GTACGAGTCT GACGAAGACA GCCTGGGCAG CTCTGGACGG    1440
GTATGTGTAG AGAAGTGGAA TCTCCTCAAC TCCTCCCGCC TCCACCTGCC GAGGGCTTCG    1500
GCCGTGGCCC TGGAAGTGCA AAGGCTTAAT GCTCTGGACC TCGAAAAGAA AATCGGGAAG    1560
TCCATTTTGG GGAAGGTCCA TCTGGCCATG GTGCGCTACC ACGAGGGTGG GCGCTTCTGC    1620
GAGAAGGGCG AGGAGTGGGA CCAGGAGTCG GCTGTCTTCC ACCTGGAGCA CGCAGCCAAC    1680
CTGGGCGAGC TGGAGGCCAT CGTGGGCCTG GGACTCATGT ACTCGCAGTT GCCTCATCAC    1740
ATCCTAGCCG ATGTCTCTCT GAAGGAGACA GAAGAGAACA AAACCAAAGG ATTTGATTAC    1800
TTACTAAAGG CCGCTGAAGC TGGCGACAGG CAGTCCATGA TCCTAGTGGC GCGAGCTTTT    1860
GACTCTGGCC AGAACCTCAG CCCGGACAGG TGCCAAGACT GGCTAGAGGC CCTGCACTGG    1920
TACAACACTG CCCTGGAGAT GACGGACTGT GATGAGGGCG GTGAGTACGA CGGAATGCAG    1980
GACGAGCCCC GGTACATGAT GCTGGCCAGG GAGGCAGAGA TGCTGTTCAC AGGAGGCTAC    2040
GGGCTGGAGA AGGACCCGCA GAGATCAGGG GACTTGTATA CCCAGGCAGC AGAGGCAGCG    2100
ATGGAAGCCA TGAAGGGCCG ACTGGCCAAC CAGTACTACC AAAAGGCTGA AGAGGCCTGG    2160
GCCCAGATGG AGGAATAA                                                  2178
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 725 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Asp Glu Asp Leu Ile Phe Arg Leu Glu Gly Val Asp Gly Gly
1 5 10 15

Gln Ser Pro Arg Ala Gly His Asp Gly Asp Ser Asp Gly Asp Ser Asp
20 25 30

Asp Glu Glu Gly Tyr Phe Ile Cys Pro Ile Thr Asp Asp Pro Ser Ser
35 40 45

Asn Gln Asn Val Asn Ser Lys Val Asn Lys Tyr Tyr Ser Asn Leu Thr
50 55 60

Lys Ser Glu Arg Tyr Ser Ser Ser Gly Ser Pro Ala Asn Ser Phe His
65 70 75 80

Phe Lys Glu Ala Trp Lys His Ala Ile Gln Lys Ala Lys His Met Pro
85 90 95

Asp Pro Trp Ala Glu Phe His Leu Glu Asp Ile Ala Thr Glu Arg Ala
100 105 110

Thr Arg His Arg Tyr Asn Ala Val Thr Gly Glu Trp Leu Asp Asp Glu
115 120 125

Val Leu Ile Lys Met Ala Ser Gln Pro Phe Gly Arg Gly Ala Met Arg
130 135 140

Glu Cys Phe Arg Thr Lys Lys Leu Ser Asn Phe Leu His Ala Gln Gln
145 150 155 160

Trp Lys Gly Ala Ser Asn Tyr Val Ala Lys Arg Tyr Ile Glu Pro Val
165 170 175

Asp Arg Asp Val Tyr Phe Glu Asp Val Arg Leu Gln Met Glu Ala Lys
180 185 190

Leu Trp Gly Glu Glu Tyr Asn Arg His Lys Pro Pro Lys Gln Val Asp
195 200 205

Ile Met Gln Met Cys Ile Ile Glu Leu Lys Asp Arg Pro Gly Lys Pro
210 215 220

Leu Phe His Leu Glu His Tyr Ile Glu Gly Lys Tyr Ile Lys Tyr Asn
225 230 235 240

Ser Asn Ser Gly Phe Val Arg Asp Asp Asn Ile Arg Leu Thr Pro Gln
245 250 255

Ala Phe Ser His Phe Thr Phe Glu Arg Ser Gly His Gln Leu Ile Val

260 265 270

Val Asp Ile Gln Gly Val Gly Asp Leu Tyr Thr Asp Pro Gln Ile His
275 280 285

Thr Glu Thr Gly Thr Asp Phe Gly Asp Gly Asn Leu Gly Val Arg Gly
290 295 300

Met Ala Leu Phe Phe Tyr Ser His Ala Cys Asn Arg Ile Cys Glu Ser
305 310 315 320

Met Gly Leu Ala Pro Phe Asp Leu Ser Pro Arg Glu Arg Asp Ala Val
325 330 335

Asn Gln Asn Thr Lys Leu Leu Gln Ser Ala Lys Thr Ile Leu Arg Gly
340 345 350

Thr Glu Glu Lys Cys Gly Ser Pro Arg Val Arg Thr Leu Ser Gly Ser
355 360 365

Arg Pro Pro Leu Leu Arg Pro Leu Ser Glu Asn Ser Gly Asp Glu Asn
370 375 380

Met Ser Asp Val Thr Phe Asp Ser Leu Pro Ser Ser Pro Ser Ser Ala
385 390 395 400

Thr Pro His Ser Gln Lys Leu Asp His Leu His Trp Pro Val Phe Ser
405 410 415

Asp Leu Asp Asn Met Ala Ser Arg Asp His Asp His Leu Asp Asn His
420 425 430

Arg Glu Ser Glu Asn Ser Gly Asp Ser Gly Tyr Pro Ser Glu Lys Arg
435 440 445

Gly Glu Leu Asp Asp Pro Glu Pro Arg Glu His Gly His Ser Tyr Ser
450 455 460

Asn Arg Lys Tyr Glu Ser Asp Glu Asp Ser Leu Gly Ser Ser Gly Arg
465 470 475 480

Val Cys Val Glu Lys Trp Asn Leu Leu Asn Ser Ser Arg Leu His Leu
485 490 495

Pro Arg Ala Ser Ala Val Ala Leu Glu Val Gln Arg Leu Asn Ala Leu
500 505 510

Asp Leu Glu Lys Lys Ile Gly Lys Ser Ile Leu Gly Lys Val His Leu
515 520 525

Ala Met Val Arg Tyr His Glu Gly Gly Arg Phe Cys Glu Lys Gly Glu

```
                530                       535                       540

        Glu Trp Asp Gln Glu Ser Ala Val Phe His Leu Glu His Ala Ala Asn
        545                     550                     555                     560

        Leu Gly Glu Leu Glu Ala Ile Val Gly Leu Gly Leu Met Tyr Ser Gln
                            565                     570                     575

        Leu Pro His His Ile Leu Ala Asp Val Ser Leu Lys Glu Thr Glu Glu
                        580                     585                     590

        Asn Lys Thr Lys Gly Phe Asp Tyr Leu Leu Lys Ala Ala Glu Ala Gly
                    595                     600                     605

        Asp Arg Gln Ser Met Ile Leu Val Ala Arg Ala Phe Asp Ser Gly Gln
            610                     615                     620

        Asn Leu Ser Pro Asp Arg Cys Gln Asp Trp Leu Glu Ala Leu His Trp
        625                     630                     635                     640

        Tyr Asn Thr Ala Leu Glu Met Thr Asp Cys Asp Glu Gly Gly Glu Tyr
                            645                     650                     655

        Asp Gly Met Gln Asp Glu Pro Arg Tyr Met Met Leu Ala Arg Glu Ala
                        660                     665                     670

        Glu Met Leu Phe Thr Gly Gly Tyr Gly Leu Glu Lys Asp Pro Gln Arg
                    675                     680                     685

        Ser Gly Asp Leu Tyr Thr Gln Ala Ala Glu Ala Ala Met Glu Ala Met
            690                     695                     700

        Lys Gly Arg Leu Ala Asn Gln Tyr Tyr Gln Lys Ala Glu Glu Ala Trp
        705                     710                     715                     720

        Ala Gln Met Glu Glu
                            725
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2175 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGGCAGACG AAGACCTCAT CTTCTGCCTG GAAGGTGTTG ACGGTGGCAG GTGCTCCCGA 60

GCTGGCCACA ATGCGGACTC TGACACAGAC AGTGACGATG ATGAGGGCTA TTTCATCTGC 120

CCCATCACTG ATGACCACAT GTCCAATCAG AATGTCAGCT CCAAAGTCCA GAGCTACTAT 180

AGCAACCTAA CAAAAACAGA GTGCGGCTCC ACAGGGTCAC CAGCCAGCTC CTTCCACTTC 240

AAGGAAGCCT GGAAGCATGC GATCGAGAAA GCCAAGCACA TGCCTGACCC CTGGGCTGAA 300

TTCCATCTCG AGGACATCGC CACAGAACAT GCTACTCGGC ACAGGTACAA CGCTGTCACC 360

GGGGAATGGC TGAAAGACGA GGTTCTGATC AAGATGGCGT CTCAGCCCTT CGGCCGTGGA 420

GCAATGAGGG AGTGCTTCAG GACGAAGAAA CTCTCCAACT TCTTGCACGC CCAGCAATGG 480

AAGGGGGCCT CCAACTACGT GGCCAAGCGC TACATCGAGC CGGTGGACAG GAGCGTGTAC 540

TTTGAGGATG TGCAGCTCCA GATGGAGGCG AAGCTCTGGG GGGAGGATTA CAATCGGCAC 600

AAGCCCCCCA AGCAGGTGGA TATCATGCAG ATGTGCATCA TTGAGCTAAA GGACAGACCA 660

GGCCAGCCCC TCTTCCACTT GGAGCACTAC ATTGAGGGCA AGTACATCAA GTACAATTCC 720

AACTCAGGCT TTGTCCGTGA TGACAACATC CGACTAACCC CACAGGCCTT CAGCCATTTC 780

ACATTTGAGC GTTCTGGTCA TCAGCTGATT GTAGTGGACA TCCAGGGTGT GGGTGACCTT 840

TATACCGACC CACAGATCCA CACTGAGAAA GGCACTGACT TTGGAGATGG TAACCTTGGT 900

GTCCGGGGAA TGGCTCTCTT CTTCTACTCT CATGCCTGCA ACCGGATTTG TCAGAGCATG 960

GGCCTTACGC CCTTTGACCT CTCCCCACGG GAACAGGATG CGGTGAATCA GAGCACCAGG 1020

CTATTGCAAT CAGCCAAGAC CATCTTGAGG GGGACAGAGG AGAAGTGTGG GAGTCCCCGC 1080

ATAAGGACAC TCTCTAGCAG CCGGCCCCCT TTGCTCCTTC GCCTGTCAGA GAACTCCGGG 1140

GATGAGAACA TGAGTGACGT GACCTTTGAC TCTCTGCCTT CCTCCCCGTC TTCAGCTACA 1200

CCACACAGCC AGAAACTGGA CCACCTCCAT TGGCCAGTGT TTGGTGACCT CGATAACATG 1260

GGCCCTAGAG ACCATGACCG TATGGACAAT CACCGGGACT CTGAGAATAG TGGGGACAGT 1320

GGGTATCCAA GCGAGAAGCG AAGTGACCTG GATGATCCTG AGCCCCGAGA ACACGGCCAC 1380

TCCAACGGCA ACCGAAGGCA TGAATCTGAC GAGGATAGCC TGGGCAGCTC TGGACGGGTC 1440

TGTGTGGAGA CGTGGAACCT GCTCAATCCC TCCCGCCTGC ACCTGCCGAG GCCCTCGGCC 1500

GTGGCCCTAG AAGTGCAGAG GCTAAATGCC CTGGACCTTG GAAGGAAAAT CGGGAAGTCT 1560

GTTTTGGGGA AAGTCCATTT GGCCATGGTG CGATACCACG AGGGCGGGCG CTTCTGCGAG 1620

AAGGATGAGG AGTGGGATCG AGAGTCAGCC ATCTTCCATC TGGAGCATGC AGCTGACCTG 1680

GGAGAACTGG AGGCCATCGT GGGCCTAGGC CTCATGTACT CTCAGCTGCC CCACCACATC 1740

CTGGCTGATG TCTCTCTGAA GGAGACAGAG GAGAACAAGA CAAAAGGCTT TGATTACTTA 1800

CTGAAGGCGG CAGAAGCTGG TGACAGGCAT TCCATGATTT TAGTGGCCCG AGCTTTTGAC 1860

ACTGGCCTGA ACCTCAGCCC AGACAGGTGT CAAGACTGGT CGGAAGCCTT GCACTGGTAC 1920

AACACAGCCC TGGAGACAAC AGACTGCGAT GAAGGCGGGG AGTACGATGG GATACAGGAC 1980

GAGCCCCAGT ACGCACTGCT GGCCAGGGAG GCGGAGATGC TGCTCACCGG GGGATTTGGA 2040

CTGGACAAGA ACCCCCAAAG ATCAGGAGAT TTGTACACCC AGGCAGCTGA GGCAGCAATG 2100

GAAGCCATGA AGGGCCGGCT AGCCAACCAG TACTACGAGA AGGCGGAAGA GGCCTGGGCC 2160

CAGATGGAGG AATAA 2175

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 724 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Asp Glu Asp Leu Ile Phe Cys Leu Glu Gly Val Asp Gly Gly
1 5 10 15

Arg Cys Ser Arg Ala Gly His Asn Ala Asp Ser Asp Thr Asp Ser Asp

20 25 30

Asp Asp Glu Gly Tyr Phe Ile Cys Pro Ile Thr Asp Asp His Met Ser
35 40 45

Asn Gln Asn Val Ser Ser Lys Val Gln Ser Tyr Tyr Ser Asn Leu Thr
50 55 60

Lys Thr Glu Cys Gly Ser Thr Gly Ser Pro Ala Ser Ser Phe His Phe
65 70 75 80

Lys Glu Ala Trp Lys His Ala Ile Glu Lys Ala Lys His Met Pro Asp
85 90 95

Pro Trp Ala Glu Phe His Leu Glu Asp Ile Ala Thr Glu His Ala Thr
100 105 110

Arg His Arg Tyr Asn Ala Val Thr Gly Glu Trp Leu Lys Asp Glu Val
115 120 125

Leu Ile Lys Met Ala Ser Gln Pro Phe Gly Arg Gly Ala Met Arg Glu
130 135 140

Cys Phe Arg Thr Lys Lys Leu Ser Asn Phe Leu His Ala Gln Gln Trp
145 150 155 160

Lys Gly Ala Ser Asn Tyr Val Ala Lys Arg Tyr Ile Glu Pro Val Asp
165 170 175

Arg Ser Val Tyr Phe Glu Asp Val Gln Leu Gln Met Glu Ala Lys Leu
180 185 190

Trp Gly Glu Asp Tyr Asn Arg His Lys Pro Pro Lys Gln Val Asp Ile
195 200 205

Met Gln Met Cys Ile Ile Glu Leu Lys Asp Arg Pro Gly Gln Pro Leu
210 215 220

Phe His Leu Glu His Tyr Ile Glu Gly Lys Tyr Ile Lys Tyr Asn Ser
225 230 235 240

Asn Ser Gly Phe Val Arg Asp Asp Asn Ile Arg Leu Thr Pro Gln Ala
245 250 255

Phe Ser His Phe Thr Phe Glu Arg Ser Gly His Gln Leu Ile Val Val
260 265 270

Asp Ile Gln Gly Val Gly Asp Leu Tyr Thr Asp Pro Gln Ile His Thr
275 280 285

Glu Lys Gly Thr Asp Phe Gly Asp Gly Asn Leu Gly Val Arg Gly Met

290 295 300

Ala Leu Phe Phe Tyr Ser His Ala Cys Asn Arg Ile Cys Gln Ser Met
305 310 315 320

Gly Leu Thr Pro Phe Asp Leu Ser Pro Arg Glu Gln Asp Ala Val Asn
325 330 335

Gln Ser Thr Arg Leu Leu Gln Ser Ala Lys Thr Ile Leu Arg Gly Thr
340 345 350

Glu Glu Lys Cys Gly Ser Pro Arg Ile Arg Thr Leu Ser Ser Ser Arg
355 360 365

Pro Pro Leu Leu Leu Arg Leu Ser Glu Asn Ser Gly Asp Glu Asn Met
370 375 380

Ser Asp Val Thr Phe Asp Ser Leu Pro Ser Ser Pro Ser Ser Ala Thr
385 390 395 400

Pro His Ser Gln Lys Leu Asp His Leu His Trp Pro Val Phe Gly Asp
405 410 415

Leu Asp Asn Met Gly Pro Arg Asp His Asp Arg Met Asp Asn His Arg
420 425 430

Asp Ser Glu Asn Ser Gly Asp Ser Gly Tyr Pro Ser Glu Lys Arg Ser
435 440 445

Asp Leu Asp Asp Pro Glu Pro Arg Glu His Gly His Ser Asn Gly Asn
450 455 460

Arg Arg His Glu Ser Asp Glu Asp Ser Leu Gly Ser Ser Gly Arg Val
465 470 475 480

Cys Val Glu Thr Trp Asn Leu Leu Asn Pro Ser Arg Leu His Leu Pro
485 490 495

Arg Pro Ser Ala Val Ala Leu Glu Val Gln Arg Leu Asn Ala Leu Asp
500 505 510

Leu Gly Arg Lys Ile Gly Lys Ser Val Leu Gly Lys Val His Leu Ala
515 520 525

Met Val Arg Tyr His Glu Gly Gly Arg Phe Cys Glu Lys Asp Glu Glu
530 535 540

Trp Asp Arg Glu Ser Ala Ile Phe His Leu Glu His Ala Ala Asp Leu
545 550 555 560

Gly Glu Leu Glu Ala Ile Val Gly Leu Gly Leu Met Tyr Ser Gln Leu

565 570 575

Pro His His Ile Leu Ala Asp Val Ser Leu Lys Glu Thr Glu Glu Asn
580 585 590

Lys Thr Lys Gly Phe Asp Tyr Leu Leu Lys Ala Ala Glu Ala Gly Asp
595 600 605

Arg His Ser Met Ile Leu Val Ala Arg Ala Phe Asp Thr Gly Leu Asn
610 615 620

Leu Ser Pro Asp Arg Cys Gln Asp Trp Ser Glu Ala Leu His Trp Tyr
625 630 635 640

Asn Thr Ala Leu Glu Thr Thr Asp Cys Asp Glu Gly Gly Glu Tyr Asp
645 650 655

Gly Ile Gln Asp Glu Pro Gln Tyr Ala Leu Leu Ala Arg Glu Ala Glu
660 665 670

Met Leu Leu Thr Gly Gly Phe Gly Leu Asp Lys Asn Pro Gln Arg Ser
675 680 685

Gly Asp Leu Tyr Thr Gln Ala Ala Glu Ala Ala Met Glu Ala Met Lys
690 695 700

Gly Arg Leu Ala Asn Gln Tyr Tyr Glu Lys Ala Glu Glu Ala Trp Ala
705 710 715 720

Gln Met Glu Glu

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3465 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Dictyostelium discoideum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGTTTAATA TAAAAAAGAG AAAAGAGAGT ATAACAGGTA TACCACCAAT AAATGTTAAT 60

AGTCCACAAT CAGTTCCATT GAGTGGAACA TTGCAATCAC CATTGATTAC ACCAAATTCA 120

CCAAATTTTG TTTCACGTCA ATGTCCATTC AAAAAGTTTG GATGTAGTAG TTTTTTAGTT 180

TCAAAGGCAG AGTTTGATAA TCACTTAAAG GATGACGCAC AATTTCATTT ACAATTGGCA 240

GTGGAGAAAT TTGATCATCA ATTTGATTTA CACACACAAT TGATGGCACA TTTTACTGAG 300

CAAATGGAGG ATCAATTAGA GAAAACAATG AAGGTCGTAC GTAATCATAC AGATAGTTTA 360

GGCGGTAATG TTCAAACCAA ATTGGATGAA GGCATTGAAA AATGTATGGC TTTTGCTAAA 420

AAGGTTGAAC AACAACAACA ACAATTGGCC AAAAGATTAA TCACTCAACA AATTCAAGAG 480

AAGAAATCAA CCTCTTCACC TTTAGTTAAA GGTGGTATTA GTGGTGGTGG TGGTAGTGGT 540

GGCGATGATT CTTTTGATGG CGCAAATATA TCATCAATGT CAACTAGTAA ACAAGAATTA 600

CAACAAGAAT TACAATCATT ATCAATTAAA ATGAAAAAAG AATTGACAGA ATTATCCGAT 660

GAACTATCAC AAAAATTAGA ACGTTCAACA GGTAATATAG ATATTAAAAT AAAGAGAATC 720

GAAGGTGAAG TTAATGAAAA GATTGATAAA CGTCAATTGG TCTCTACGAT CGATGATTCA 780

ATTGGAAAGA AAACAGATTC CATCGGTTAT ACATTGGAGA GTTCAATCAT TAAAAAGGTT 840

GAAGAGAAAG AGAAAAAGAA ATCCGAACAA AATCAACTTC TCTTTGATTC AAAGATTGAA 900

TCCTTAAAAG ATAAGATTAA AATCATTGAA ACTCAACAAT TGGATACTTC ATCAGAGGTT 960

AGAAAATTGA AATTAGAAAG TACAAGTAGT GGAAATTTAA TGGCAGGTCT TAATGGTACC 1020

TCTGGTAGAC CTTCATCATC TTCTCACTTT ATTCCATCCT CTGTTTCTGC CGCTGCTAAC 1080

AATATCAACA AGAATGAAAT CATGGAAGAG GTTAAAAAGG TAGAAGAGAA ACTTCAAAAG 1140

AAAATTCGTG AAGAGATTGA TAATACAAAA GCTGAACTCT CAAAGGTTGA ACGTTCCGTT 1200

AAAGATAATC GTAGTGAAAT TGAAGGTTTG GAAAAAGATT GTAAGAATCA ATTCGATAAA 1260

CAAGACAATA AGATCAAACA AGTTGAGGAT GATTTGAAAA AGAGTGATTC ATTACTTTTG 1320

TTAATGCAAA ATAACCTCAA GAAATATAAT GAATTTGTTG ATAGAGAACG TGATCGTGAA 1380

AGTGAACGTT TGAAACTTCA AGATTCTATC AAACGTTTAG AACAAAATCA AAAGAAAATC 1440

GAAGCTGAAA TTCAAGAAGG TAATGAACAA GTTGAACGTG TTTTACGTGA GGAAGCTTCA 1500

ATCTCACCAA TTAGTTCAGT TCCAAAATCA CCAATCACAA CCAAACGTTC ATCGATTATT 1560

TTAAATTCAC CACCAATGAC TTCACAACAA TCATCACCAA AGATTCAAGA TCTTCTCTCA 1620

AGTAGTGGTA GTAGTAGTGT TAGTGGTATA AATATTTCCT CTGAAACCGG TGAAATGGGT 1680

ATTCTTTGGG AATTTGATCC AATCATTAAC AAATGGATTA GATTATCAAT GAAGCTAAAG 1740

GTAGAAAGAA AACCATTTGC AGAGGGTGCT CTTAGAGAGG CTTATCATAC CGTTTCATTG 1800

GGTGTTGGAA CCGATGAAAA TTATCCATTA GGTACAACCA CCAAATTATT CCCACCAATT 1860

GAAATGATTT CACCAATTTC AAAGAATAAT GAGGCAATGA CTCAATTGAA GAATGGTACA 1920

AAATTTGTTT TGAAACTCTA CAAAAAGGAA GCTGAACAAC AAGCTAGCAG AGAATTATAC 1980

TTTGAAGATG TTAAAATGCA AATGGTCTGT AGAGATTGGG GTAATAAATT CAATCAAAAG 2040

AAACCACCAA AGAAAATTGA ATTCCTTATG TCTTGGGTTG TAGAGTTAAT CGATAGATCT 2100

CCTTCTTCCA ATGGTCAACC AATACTTTGT TCCATTGAAC CATTATTGGT TGGTGAATTC 2160

AAAAAGAATA ATTCAAATTA TGGTGCAGTT TTAACCAATC GTTCAACTCC ACAAGCATTC 2220

TCTCATTTCA CCTATGAACT CTCAAATAAA CAAATGATCG TTGTCGATAT TCAAGGTGTT 2280

GATGATCTTT ACACTGATCC TCAAATTCAT ACACCCGATG GTAAAGGATT TGGTCTTGGT 2340

AATCTTGGTA AAGCAGGTAT CAATAAATTC ATCACCACTC ACAAATGTAA TGCTGTTTGT 2400

GCTCTTTTAG ATTTAGATGT TAAATTGGGT GGTGTACTAT CTGGAAATAA TAAGAAACAA 2460

CTTCAACAAG GTACTATGGT TATGCCAGAT ATTCTCCCAG AACTTATGCC ATCTGATAAC 2520

ACCATTAAAG TGGGTGCAAA ACAACTTCCA AAAGCTGAAT TCTCAAAGAA AGATCTCAAA 2580

TGTGTTAGCA CCATTCAAAG TTTCCGTGAA CGTGTTAACT CGATCGCATT CTTTGATAAT 2640

CAAAAGTTAT TATGCGCTGG TTATGGTGAT GGTACCTATA GAGTTTTCGA TGTCAATGAC 2700

AATTGGAAAT GTTTATACAC TGTCAATGGT CATAGAAAAT CAATTGAAAG TATCGCTTGT 2760

AATAGTAATT ACATTTTCAC TTCATCACCT GATAACACCA TCAAAGTTCA TATCATTCGT 2820

AGTGGTAACA CCAAATGTAT AGAGACATTG GTTGGTCACA CTGGTGAAGT TAATTGTGTC 2880

GTGGCCAATG AAAAATATCT TTTCAGTTGT AGTTATGATA AAACTATCAA GGTTTGGGAT 2940

TTGTCAACCT TTAAAGAAAT TAAATCATTT GAGGGTGTTC ATACAAAGTA CATTAAAACA 3000

TTGGCTTTGA GTGGACGTTA TCTTTTTAGT GGTGGTAACG ATCAAATCAT TTACGTTTGG 3060

GATACTGAAA CACTTAGTAT GCTTTTCAAT ATGCAAGGTC ATGAAGATTG GGTACTCTCT 3120

CTTCATTGTA CCGCTAGTTA TCTTTTCTCA ACCTCAAAAG ATAATGTCAT CAAGATTTGG 3180

GATCTCTCAA ATTTCAGTTG TATCGATACT CTAAAAGGTC ATTGGAATTC TGTCTCAAGT 3240

TGTGTCGTAA AAGATCGTTA TCTATACAGT GGTTCTGAAG ATAATTCAAT CAAAGTTTGG 3300

GATCTCGATA CACTTGAATG TGTTTACACC ATTCCAAAAT CTCATTCTTT GGGTGTAAAA 3360

TGTTTAATGG TTTTCAATAA TCAAATCATT TCTGCTGCTT TCGATGGTTC AATTAAAGTT 3420

TGGGAATGGC AATCGAAATA ATCTTTGTAA ATTTTTGTTA AAAAA 3465

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1146 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Dictyostelium discoideum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Phe Asn Ile Lys Lys Arg Lys Glu Ser Ile Thr Gly Ile Pro Pro
1 5 10 15

Ile Asn Val Asn Ser Pro Gln Ser Val Pro Leu Ser Gly Thr Leu Gln
20 25 30

Ser Pro Leu Ile Thr Pro Asn Ser Pro Asn Phe Val Ser Arg Gln Cys
35 40 45

Pro Phe Lys Lys Phe Gly Cys Ser Ser Phe Leu Val Ser Lys Ala Glu
50 55 60

Phe Asp Asn His Leu Lys Asp Asp Ala Gln Phe His Leu Gln Leu Ala
65 70 75 80

Val Glu Lys Phe Asp His Gln Phe Asp Leu His Thr Gln Leu Met Ala
85 90 95

His Phe Thr Glu Gln Met Glu Asp Gln Leu Glu Lys Thr Met Lys Val
100 105 110

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Val Arg Asn His Thr Asp Ser Leu Gly Gly Asn Val Gln Thr Lys Leu
        115                 120                 125

Asp Glu Gly Ile Glu Lys Cys Met Ala Phe Ala Lys Lys Val Glu Gln
    130                 135                 140

Gln Gln Gln Gln Leu Ala Lys Arg Leu Ile Thr Gln Gln Ile Gln Glu
145                 150                 155                 160

Lys Lys Ser Thr Ser Ser Pro Leu Val Lys Gly Gly Ile Ser Gly Gly
                165                 170                 175

Gly Gly Ser Gly Gly Asp Asp Ser Phe Asp Gly Ala Asn Ile Ser Ser
            180                 185                 190

Met Ser Thr Ser Lys Gln Glu Leu Gln Gln Glu Leu Gln Ser Leu Ser
        195                 200                 205

Ile Lys Met Lys Lys Glu Leu Thr Glu Leu Ser Asp Glu Leu Ser Gln
    210                 215                 220

Lys Leu Glu Arg Ser Thr Gly Asn Ile Asp Ile Lys Ile Lys Arg Ile
225                 230                 235                 240

Glu Gly Glu Val Asn Glu Lys Ile Asp Lys Arg Gln Leu Val Ser Thr
                245                 250                 255

Ile Asp Asp Ser Ile Gly Lys Lys Thr Asp Ser Ile Gly Tyr Thr Leu
            260                 265                 270

Glu Ser Ser Ile Ile Lys Lys Val Glu Glu Lys Glu Lys Lys Lys Ser
        275                 280                 285

Glu Gln Asn Gln Leu Leu Phe Asp Ser Lys Ile Glu Ser Leu Lys Asp
    290                 295                 300

Lys Ile Lys Ile Ile Glu Thr Gln Gln Leu Asp Thr Ser Ser Glu Val
305                 310                 315                 320

Arg Lys Leu Lys Leu Glu Ser Thr Ser Ser Gly Asn Leu Met Ala Gly
                325                 330                 335

Leu Asn Gly Thr Ser Gly Arg Pro Ser Ser Ser Ser His Phe Ile Pro
            340                 345                 350

Ser Ser Val Ser Ala Ala Ala Asn Asn Ile Asn Lys Asn Glu Ile Met
        355                 360                 365

Glu Glu Val Lys Lys Val Glu Glu Lys Leu Gln Lys Lys Ile Arg Glu
    370                 375                 380
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Glu	Ile	Asp	Asn	Thr	Lys	Ala	Glu	Leu	Ser	Lys	Val	Glu	Arg	Ser	Val
385					390					395					400
Lys	Asp	Asn	Arg	Ser	Glu	Ile	Glu	Gly	Leu	Glu	Lys	Asp	Cys	Lys	Asn
				405					410					415	
Gln	Phe	Asp	Lys	Gln	Asp	Asn	Lys	Ile	Lys	Gln	Val	Glu	Asp	Asp	Leu
			420					425					430		
Lys	Lys	Ser	Asp	Ser	Leu	Leu	Leu	Leu	Met	Gln	Asn	Asn	Leu	Lys	Lys
		435					440					445			
Tyr	Asn	Glu	Phe	Val	Asp	Arg	Glu	Arg	Asp	Arg	Glu	Ser	Glu	Arg	Leu
	450					455					460				
Lys	Leu	Gln	Asp	Ser	Ile	Lys	Arg	Leu	Glu	Gln	Asn	Gln	Lys	Lys	Ile
465					470					475					480
Glu	Ala	Glu	Ile	Gln	Glu	Gly	Asn	Glu	Gln	Val	Glu	Arg	Val	Leu	Arg
				485					490					495	
Glu	Glu	Ala	Ser	Ile	Ser	Pro	Ile	Ser	Ser	Val	Pro	Lys	Ser	Pro	Ile
			500					505					510		
Thr	Thr	Lys	Arg	Ser	Ser	Ile	Ile	Leu	Asn	Ser	Pro	Pro	Met	Thr	Ser
		515					520					525			
Gln	Gln	Ser	Ser	Pro	Lys	Ile	Gln	Asp	Leu	Leu	Ser	Ser	Ser	Gly	Ser
	530					535					540				
Ser	Ser	Val	Ser	Gly	Ile	Asn	Ile	Ser	Ser	Glu	Thr	Gly	Glu	Met	Gly
545					550					555					560
Ile	Leu	Trp	Glu	Phe	Asp	Pro	Ile	Ile	Asn	Lys	Trp	Ile	Arg	Leu	Ser
				565					570					575	
Met	Lys	Leu	Lys	Val	Glu	Arg	Lys	Pro	Phe	Ala	Glu	Gly	Ala	Leu	Arg
			580					585					590		
Glu	Ala	Tyr	His	Thr	Val	Ser	Leu	Gly	Val	Gly	Thr	Asp	Glu	Asn	Tyr
		595					600					605			
Pro	Leu	Gly	Thr	Thr	Thr	Lys	Leu	Phe	Pro	Pro	Ile	Glu	Met	Ile	Ser
	610					615					620				
Pro	Ile	Ser	Lys	Asn	Asn	Glu	Ala	Met	Thr	Gln	Leu	Lys	Asn	Gly	Thr
625					630					635					640
Lys	Phe	Val	Leu	Lys	Leu	Tyr	Lys	Lys	Glu	Ala	Glu	Gln	Gln	Ala	Ser
				645					650					655	

Arg Glu Leu Tyr Phe Glu Asp Val Lys Met Gln Met Val Cys Arg Asp
660 665 670

Trp Gly Asn Lys Phe Asn Gln Lys Lys Pro Pro Lys Lys Ile Glu Phe
675 680 685

Leu Met Ser Trp Val Val Glu Leu Ile Asp Arg Ser Pro Ser Ser Asn
690 695 700

Gly Gln Pro Ile Leu Cys Ser Ile Glu Pro Leu Leu Val Gly Glu Phe
705 710 715 720

Lys Lys Asn Asn Ser Asn Tyr Gly Ala Val Leu Thr Asn Arg Ser Thr
725 730 735

Pro Gln Ala Phe Ser His Phe Thr Tyr Glu Leu Ser Asn Lys Gln Met
740 745 750

Ile Val Val Asp Ile Gln Gly Val Asp Asp Leu Tyr Thr Asp Pro Gln
755 760 765

Ile His Thr Pro Asp Gly Lys Gly Phe Gly Leu Gly Asn Leu Gly Lys
770 775 780

Ala Gly Ile Asn Lys Phe Ile Thr Thr His Lys Cys Asn Ala Val Cys
785 790 795 800

Ala Leu Leu Asp Leu Asp Val Lys Leu Gly Gly Val Leu Ser Gly Asn
805 810 815

Asn Lys Lys Gln Leu Gln Gln Gly Thr Met Val Met Pro Asp Ile Leu
820 825 830

Pro Glu Leu Met Pro Ser Asp Asn Thr Ile Lys Val Gly Ala Lys Gln
835 840 845

Leu Pro Lys Ala Glu Phe Ser Lys Lys Asp Leu Lys Cys Val Ser Thr
850 855 860

Ile Gln Ser Phe Arg Glu Arg Val Asn Ser Ile Ala Phe Phe Asp Asn
865 870 875 880

Gln Lys Leu Leu Cys Ala Gly Tyr Gly Asp Gly Thr Tyr Arg Val Phe
885 890 895

Asp Val Asn Asp Asn Trp Lys Cys Leu Tyr Thr Val Asn Gly His Arg
900 905 910

Lys Ser Ile Glu Ser Ile Ala Cys Asn Ser Asn Tyr Ile Phe Thr Ser
915 920 925

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Ser Pro Asp Asn Thr Ile Lys Val His Ile Ile Arg Ser Gly Asn Thr
    930                 935                     940

Lys Cys Ile Glu Thr Leu Val Gly His Thr Gly Glu Val Asn Cys Val
945                 950                     955                 960

Val Ala Asn Glu Lys Tyr Leu Phe Ser Cys Ser Tyr Asp Lys Thr Ile
                965                 970                 975

Lys Val Trp Asp Leu Ser Thr Phe Lys Glu Ile Lys Ser Phe Glu Gly
            980                 985                 990

Val His Thr Lys Tyr Ile Lys Thr Leu Ala Leu Ser Gly Arg Tyr Leu
        995                 1000                1005

Phe Ser Gly Gly Asn Asp Gln Ile Ile Tyr Val Trp Asp Thr Glu Thr
    1010                1015                1020

Leu Ser Met Leu Phe Asn Met Gln Gly His Glu Asp Trp Val Leu Ser
1025                1030                1035                1040

Leu His Cys Thr Ala Ser Tyr Leu Phe Ser Thr Ser Lys Asp Asn Val
                1045                1050                1055

Ile Lys Ile Trp Asp Leu Ser Asn Phe Ser Cys Ile Asp Thr Leu Lys
            1060                1065                1070

Gly His Trp Asn Ser Val Ser Ser Cys Val Val Lys Asp Arg Tyr Leu
        1075                1080                1085

Tyr Ser Gly Ser Glu Asp Asn Ser Ile Lys Val Trp Asp Leu Asp Thr
    1090                1095                1100

Leu Glu Cys Val Tyr Thr Ile Pro Lys Ser His Ser Leu Gly Val Lys
1105                1110                1115                1120

Cys Leu Met Val Phe Asn Asn Gln Ile Ile Ser Ala Ala Phe Asp Gly
                1125                1130                1135

Ser Ile Lys Val Trp Glu Trp Gln Ser Lys
            1140                1145
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2237 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Dictyostelium discoideum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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ATAAGAAGAT AGAAGATGAT ATTTAAAGTT TGGTTTTCAT ATGAAGATGA GGAAGTGGAA    60
CTATCAGAAT TAACAAATGA TACAACAGTG TCAGCAATTA GAAAGATCTT ACATGAAGGT   120
AAAATATTTA GATTTCCATA TGGTACATCT CAAACAGACT TGCAAATTGG AAAGATGTTA   180
CCATCTGGTA GTGGTGGAGG TGCAACTGCA GACAGCAAAT TTGAGAAGTT TAAAGCACGT   240
AATACATTAG CAGATATTCA ATATAAAGTT GGTGATACAT TATATGTTAG AGTTAAAAAA   300
AGTAAACCAA CAAATGATTC ATTATTACCA ACATTAAATA TAGCATTTTT AGATGGATCA   360
GAACGTGCAA TTAAATGGGA ATATGACCCA TATACTACAA CTGCTCAATG GACCTGTACA   420
GCAACATTAG TCAAAGTTGA ACCAGTACCA TTTGCTGAAG GTGCATTTAG GAAAGCTTAT   480
CATACATTGG ATTTAAGTAA ATCTGGTGCA AGTGGAAGAT ATGTATCAAA GATTGGTAAA   540
AAACCAACAC CAAGACCATC ATATTTTGAA GATGTAAAGA TGCAAATGAT AGCAAAGAAA   600
TGGGCAGATA AATATAATTC ATTTAAACCT CCAAAAAAGA TTGAATTTTT ACAATCATGC   660
GTTTTAGAGT TTGTAGATAG AACATCATCA GATTTAATTT GTGGAGCAGA ACCATATGTA   720
GAAGGACAAT ATAGAAAGTA TAATAATAAT AGTGGATTCG TTAGTAATGA TGAAAGAAAT   780
ACACCACAAT CATTCTCTCA TTTCACATAT GAACATTCAA ATCATCAATT ATTGATTATA   840
GATATTCAAG GTGTTGGTGA TCACTATACA GACCCACAAA TTCATACCTA TGATGGTGTT   900
GGTTTTGGTA TTGGTAATTT GGGTCAAAAA GGTTTTGAAA AGTTTTTAGA TACTCATAAA   960
TGTAATGCAA TTTGCCAATA TTTAAATTTA CAATCAATTA ATCCAAAATC TGAAAAAAGT  1020
GATTGTGGTA CTGTACCAAG ACCAGATTTA ATTTTCCCTG ATACATCTGA AAGAGATAAT  1080
AATAATAATA ATAATAATAA TAATAATAAT AATAATAATA ATAATAATAA TAATAGTAAT  1140
AATAATAATA ATAACAATAG TAGTATTTCA AAATCATTAG TTGAAATTTC AAGTGGTAGT  1200
AAAGAAAGAA ATGATAGAGA TTCGCCAAGT AGACAATTAT TTGTTTCAAA TGATGGTAAT  1260
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ACATTAAATA CAAATAAAGA GAGATCAAAA TCAAAATCAA TAGATTTAGA AAAACCAGAA 1320

ATTTTAATAA ATAATAAGAA AAAAGAGAGT ATAAATTTGG AAACGATAAA ATTAATTGAA 1380

ACTATTAAAG GATATCATGT TACAAGTCAT TTATGTATTT GTGATAATTT ATTATTTACA 1440

GGATGTTCAG ATAATTCAAT TAGAGTGTAT GATTATAAGA GTCAAAATAT GGAATGTGTT 1500

CAAACCTTGA AAGGTCATGA AGGTCCAGTT GAATCAATTT GTTATAATGA TCAATATTTG 1560

TTTAGTGGTT CATCAGATCA TTCAATTAAA GTTTGGGATT TAAAGAAATT AAGATGTATT 1620

TTTACTTTGG AGGGTCATGA TAAACCTGTC CATACGGTTC TATTGAATGA TAAATATTTG 1680

TTTAGTGGTT CCTCTGACAA AACTATCAAA GTTTGGGATT TGAAAACTTT GGAATGTAAA 1740

TATACCCTTG AAAGTCATGC CAGAGCCGTC AAAACACTTT GTATATCTGG TCAATATTTA 1800

TTTAGTGGTT CAAATGATAA AACTATCAAG GTTTGGGATT TGAAAACTTT TCGTTGTAAC 1860

TACACTCTAA AAGGTCATAC TAAATGGGTC ACCACTATCT GTATATTAGG TACCAATCTC 1920

TACAGTGGCT CCTATGATAA AACTATAAGA GTTTGGAATT TAAAGAGTTT AGAATGTTCC 1980

GCTACTTTAA GAGGCCATGA TAGATGGGTT GAACATATGG TAATTTGTGA TAAATTATTA 2040

TTTACTGCTA GTGACGATAA TACAATTAAA ATTTGGGATT TAGAAACATT AAGATGTAAT 2100

ACAACTTTGG AAGGACATAA TGCAACCGTT CAATGTTTAG CAGTTTGGGA AGATAAAAAA 2160

TGTGTTATTA GTTGTAGTCA TGATCAAAGT ATTAGAGTTT GGGGTTGGAA TTAATTTAAA 2220

ATAAAAAAAA AAAACAT 2237

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 732 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Dictyostelium discoideum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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Met Ile Phe Lys Val Trp Phe Ser Tyr Glu Asp Glu Glu Val Glu Leu
1               5                   10                  15

Ser Glu Leu Thr Asn Asp Thr Thr Val Ser Ala Ile Arg Lys Ile Leu
            20                  25                  30

His Glu Gly Lys Ile Phe Arg Phe Pro Tyr Gly Thr Ser Gln Thr Asp
        35                  40                  45

Leu Gln Ile Gly Lys Met Leu Pro Ser Gly Ser Gly Gly Gly Ala Thr
    50                  55                  60

Ala Asp Ser Lys Phe Glu Lys Phe Lys Ala Arg Asn Thr Leu Ala Asp
65                  70                  75                  80

Ile Gln Tyr Lys Val Gly Asp Thr Leu Tyr Val Arg Val Lys Lys Ser
                85                  90                  95

Lys Pro Thr Asn Asp Ser Leu Leu Pro Thr Leu Asn Ile Ala Phe Leu
            100                 105                 110

Asp Gly Ser Glu Arg Ala Ile Lys Trp Glu Tyr Asp Pro Tyr Thr Thr
        115                 120                 125

Thr Ala Gln Trp Thr Cys Thr Ala Thr Leu Val Lys Val Glu Pro Val
    130                 135                 140

Pro Phe Ala Glu Gly Ala Phe Arg Lys Ala Tyr His Thr Leu Asp Leu
145                 150                 155                 160

Ser Lys Ser Gly Ala Ser Gly Arg Tyr Val Ser Lys Ile Gly Lys Lys
                165                 170                 175

Pro Thr Pro Arg Pro Ser Tyr Phe Glu Asp Val Lys Met Gln Met Ile
            180                 185                 190

Ala Lys Lys Trp Ala Asp Lys Tyr Asn Ser Phe Lys Pro Pro Lys Lys
        195                 200                 205

Ile Glu Phe Leu Gln Ser Cys Val Leu Glu Phe Val Asp Arg Thr Ser
    210                 215                 220

Ser Asp Leu Ile Cys Gly Ala Glu Pro Tyr Val Glu Gly Gln Tyr Arg
225                 230                 235                 240

Lys Tyr Asn Asn Asn Ser Gly Phe Val Ser Asn Asp Glu Arg Asn Thr
                245                 250                 255

Pro Gln Ser Phe Ser His Phe Thr Tyr Glu His Ser Asn His Gln Leu
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            260                 265                 270

Leu Ile Ile Asp Ile Gln Gly Val Gly Asp His Tyr Thr Asp Pro Gln
        275                 280                 285

Ile His Thr Tyr Asp Gly Val Gly Phe Gly Ile Gly Asn Leu Gly Gln
    290                 295                 300

Lys Gly Phe Glu Lys Phe Leu Asp Thr His Lys Cys Asn Ala Ile Cys
305                 310                 315                 320

Gln Tyr Leu Asn Leu Gln Ser Ile Asn Pro Lys Ser Glu Lys Ser Asp
                325                 330                 335

Cys Gly Thr Val Pro Arg Pro Asp Leu Ile Phe Pro Asp Thr Ser Glu
            340                 345                 350

Arg Asp Asn Asn Asn Asn Asn Asn Asn Asn Asn Asn Asn Asn Asn Asn
        355                 360                 365

Asn Asn Asn Asn Asn Ser Asn Asn Asn Asn Asn Asn Asn Ser Ser Ile
    370                 375                 380

Ser Lys Ser Leu Val Glu Ile Ser Ser Gly Ser Lys Glu Arg Asn Asp
385                 390                 395                 400

Arg Asp Ser Pro Ser Arg Gln Leu Phe Val Ser Asn Asp Gly Asn Thr
                405                 410                 415

Leu Asn Thr Asn Lys Glu Arg Ser Lys Ser Lys Ser Ile Asp Leu Glu
            420                 425                 430

Lys Pro Glu Ile Leu Ile Asn Asn Lys Lys Lys Glu Ser Ile Asn Leu
        435                 440                 445

Glu Thr Ile Lys Leu Ile Glu Thr Ile Lys Gly Tyr His Val Thr Ser
    450                 455                 460

His Leu Cys Ile Cys Asp Asn Leu Leu Phe Thr Gly Cys Ser Asp Asn
465                 470                 475                 480

Ser Ile Arg Val Tyr Asp Tyr Lys Ser Gln Asn Met Glu Cys Val Gln
                485                 490                 495

Thr Leu Lys Gly His Glu Gly Pro Val Glu Ser Ile Cys Tyr Asn Asp
            500                 505                 510

Gln Tyr Leu Phe Ser Gly Ser Ser Asp His Ser Ile Lys Val Trp Asp
        515                 520                 525

Leu Lys Lys Leu Arg Cys Ile Phe Thr Leu Glu Gly His Asp Lys Pro
```

530 535 540

Val His Thr Val Leu Leu Asn Asp Lys Tyr Leu Phe Ser Gly Ser Ser
545 550 555 560

Asp Lys Thr Ile Lys Val Trp Asp Leu Lys Thr Leu Glu Cys Lys Tyr
565 570 575

Thr Leu Glu Ser His Ala Arg Ala Val Lys Thr Leu Cys Ile Ser Gly
580 585 590

Gln Tyr Leu Phe Ser Gly Ser Asn Asp Lys Thr Ile Lys Val Trp Asp
595 600 605

Leu Lys Thr Phe Arg Cys Asn Tyr Thr Leu Lys Gly His Thr Lys Trp
610 615 620

Val Thr Thr Ile Cys Ile Leu Gly Thr Asn Leu Tyr Ser Gly Ser Tyr
625 630 635 640

Asp Lys Thr Ile Arg Val Trp Asn Leu Lys Ser Leu Glu Cys Ser Ala
645 650 655

Thr Leu Arg Gly His Asp Arg Trp Val Glu His Met Val Ile Cys Asp
660 665 670

Lys Leu Leu Phe Thr Ala Ser Asp Asp Asn Thr Ile Lys Ile Trp Asp
675 680 685

Leu Glu Thr Leu Arg Cys Asn Thr Thr Leu Glu Gly His Asn Ala Thr
690 695 700

Val Gln Cys Leu Ala Val Trp Glu Asp Lys Lys Cys Val Ile Ser Cys
705 710 715 720

Ser His Asp Gln Ser Ile Arg Val Trp Gly Trp Asn
725 730

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2307 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: C. elegans

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGACGATCG ACACAACAAA TGAGAGCGAC AATAGTCCAA CTAACTCACC AGGATTGGAG 60

GCCTCGGCTC GGACATTCTC GCTCAATGCG TCAAAAATGG TTCGGATAAC CGACGACTAC 120

GCAGATGAAG TGTTCATTGA ACAGAATGAT GTCGTTATCG AGAAGCCTCG TATGGATCCT 180

CTCCACGTTA GAAAACTTAT GGAGACATGG CGCAAGGCTG CTCGCCGAGC AAGAACAAAC 240

TATATAGATC CATGGGATGA GTTCAACATC CACGAGTATC CAGTACAACG AGCTAAACGA 300

TATAGGTATT CTGCAATCAG AAAGCAATGG ACAGAGGATA TAGTCGATGT GAGACTTCAT 360

CCGGACAGTT TTGCACGTGG AGCCATGCGA GAATGCTACC GACTCAAAAA GTGCTCCAAG 420

CACGGAACAA GTCAAGATTG GAGCAGCAAC TATGTCGCAA AAAGATACAT TTGTCAAGTC 480

GATCGTAGAG TTCTTTTCGA TGATGTCAGA CTTCAGATGG ATGCCAAATT ATGGGCTGAA 540

GAATATAATC GGTATAATCC ACCGAAGAAA ATTGATATTG TTCAAATGTG TGTCATTGAG 600

ATGATTGATG TAAAAGGTTC TCCACTCTAT CATTTGGAGC ATTTCATCGA GGGAAAATAT 660

ATAAAATACA ATTCAAACTC AGGATTTGTA TCAAATGCAG CTCGTCTTAC ACCACAAGCA 720

TTTTCTCACT TCACCTTCGA ACGTTCTGGT CATCAAATGA TGGTTGTCGA TATTCAAGGA 780

GTTGGTGATC TTTACACAGA TCCTCAGATT CATACAGTTG TGGGAACTGA TTATGGAGAT 840

GGAAACCTCG GAACTCGTGG AATGGCTCTT TTCTTCCATT CACACAGATG TAACGATATT 900

TGTGAGACAA TGGATCTATC AAATTTCGAA CTTTCGCCAC CTGAAATCGA GGCTACCGAA 960

GTTGCGATGG AAGTAGCTGC AAAGCAGAAA AAGTCATGCA TAGTTCCTCC AACTGTGTTC 1020

GAAGCAAGAA GAAATCGAAT TTCAAGTGAA TGTGTACATG TCGAGCATGG TATTTCGATG 1080

GATCAATTGA GAAAAAGGAA GACGTTGAAT CAATCGTCAA CCGATTTGTC AGCAAAGAGT 1140

CACAACGAAG ACTGTGTATG TCCTGAGTGT ATTCCAGTTG TTGAGCAACT CTGTGAGCCT 1200

TGCTCCGAAG ATGAAGAGGA CGAAGAAGAA GACTATCCAA GAAGTGAAAA AAGTGGAAAT 1260

AGTCAGAAAA GTCGACGTAG TAGAATGAGC ATTTCAACGA GATCTTCTGG CGATGAATCA 1320

GCATCTCGTC CTAGAAAATG CGGATTTGTA GATTTAAACT CACTTCGTCA GAGACATGAT 1380

AGCTTCAGAA GTTCTGTTGG GACATATTCT ATGAATAGTT CTAGACAAAC CAGAGACACT 1440

GAAAAGGATG AATTCTGGAA GGTTCTTCGA AAACAATCAG TTCCAGCAAA CATTCTATCA 1500

CTTCAACTTC AACAAATGGC TGCTAACCTG GAAAATGATG AAGACGTACC ACAAGTCACC 1560

GGGCATCAGT TCTCTGTCCT CGGTCAGATT CATATTGATC TCTCACGATA TCATGAGCTC 1620

GGGCGGTTCG TAGAAGTTGA TTCAGAACAT AAGGAAATGC TTGAGGGAAG TGAAAATGAC 1680

GCTCGTGTAC CAATCAAATA CGACAAGCAG TCTGCAATTT TCCATTTGGA TATCGCTCGG 1740

AAGTGTGGAA TCCTTGAGGC TGTGCTAACA TCGGCTCATA TTGTTCTCGG ATTACCACAT 1800

GAATTGTTGA AAGAAGTCAC CGTTGATGAT CTGTTTCCTA ATGGGTTTGG AGAACAGGAA 1860

AATGGAATTC GAGCTGATAA AGGACAAAAA CCTTGTGACC TAGAAGAGTT CGGCTCCGAT 1920

CTGATGGAAA TTGCTGCAGA GATGGGTGAT AAGGGTGCAA TGCTGTACAT GGCACACGCT 1980

TATGAAACTG GTCAGCATCT CGGACCGAAT CGAAGAACGG ATTATAAGAA ATCGATTGAT 2040

TGGTATCAAC GCGTCGTTGG ATTCCAAGAA GAAGAAGAAC TTGACTCTGA TTGTGGAAAA 2100

ACGACATTCT CCTCATTTGC TCCACTGACT CGTCACGAGA TTCTAGCCAA AATGGCTGAA 2160

ATGTACAAAG AGGGAGGTTA TGGCCTGAAT CAAGACTTCG AACGAGCATA TGGTCTATTC 2220

AATGAAGCTG CTGAAGCAGC AATGGAAGCA ATGAATGGAA AGCTCGCAAA TAAATACTAT 2280

GAAAAAGCGG AAATGTGTGG AGAATGA 2307

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 768 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: C. elegans

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Thr Ile Asp Thr Thr Asn Glu Ser Asp Asn Ser Pro Thr Asn Ser
1 5 10 15

Pro Gly Leu Glu Ala Ser Ala Arg Thr Phe Ser Leu Asn Ala Ser Lys
20 25 30

Met Val Arg Ile Thr Asp Asp Tyr Ala Asp Glu Val Phe Ile Glu Gln
35 40 45

Asn Asp Val Val Ile Glu Lys Pro Arg Met Asp Pro Leu His Val Arg
50 55 60

Lys Leu Met Glu Thr Trp Arg Lys Ala Ala Arg Arg Ala Arg Thr Asn
65 70 75 80

Tyr Ile Asp Pro Trp Asp Glu Phe Asn Ile His Glu Tyr Pro Val Gln
85 90 95

Arg Ala Lys Arg Tyr Arg Tyr Ser Ala Ile Arg Lys Gln Trp Thr Glu
100 105 110

Asp Ile Val Asp Val Arg Leu His Pro Asp Ser Phe Ala Arg Gly Ala
115 120 125

Met Arg Glu Cys Tyr Arg Leu Lys Lys Cys Ser Lys His Gly Thr Ser
130 135 140

Gln Asp Trp Ser Ser Asn Tyr Val Ala Lys Arg Tyr Ile Cys Gln Val
145 150 155 160

Asp Arg Arg Val Leu Phe Asp Asp Val Arg Leu Gln Met Asp Ala Lys
165 170 175

Leu Trp Ala Glu Glu Tyr Asn Arg Tyr Asn Pro Pro Lys Lys Ile Asp
180 185 190

Ile Val Gln Met Cys Val Ile Glu Met Ile Asp Val Lys Gly Ser Pro
195 200 205

Leu Tyr His Leu Glu His Phe Ile Glu Gly Lys Tyr Ile Lys Tyr Asn
210 215 220

Ser Asn Ser Gly Phe Val Ser Asn Ala Ala Arg Leu Thr Pro Gln Ala
225 230 235 240

Phe Ser His Phe Thr Phe Glu Arg Ser Gly His Gln Met Met Val Val
245 250 255

Asp Ile Gln Gly Val Gly Asp Leu Tyr Thr Asp Pro Gln Ile His Thr
260 265 270

Val Val Gly Thr Asp Tyr Gly Asp Gly Asn Leu Gly Thr Arg Gly Met
275 280 285

Ala Leu Phe Phe His Ser His Arg Cys Asn Asp Ile Cys Glu Thr Met
290 295 300

Asp Leu Ser Asn Phe Glu Leu Ser Pro Pro Glu Ile Glu Ala Thr Glu
305 310 315 320

Val Ala Met Glu Val Ala Ala Lys Gln Lys Lys Ser Cys Ile Val Pro
325 330 335

Pro Thr Val Phe Glu Ala Arg Arg Asn Arg Ile Ser Ser Glu Cys Val
340 345 350

His Val Glu His Gly Ile Ser Met Asp Gln Leu Arg Lys Arg Lys Thr
355 360 365

Leu Asn Gln Ser Ser Thr Asp Leu Ser Ala Lys Ser His Asn Glu Asp
370 375 380

Cys Val Cys Pro Glu Cys Ile Pro Val Val Glu Gln Leu Cys Glu Pro
385 390 395 400

Cys Ser Glu Asp Glu Glu Asp Glu Glu Glu Asp Tyr Pro Arg Ser Glu
405 410 415

Lys Ser Gly Asn Ser Gln Lys Ser Arg Arg Ser Arg Met Ser Ile Ser
420 425 430

Thr Arg Ser Ser Gly Asp Glu Ser Ala Ser Arg Pro Arg Lys Cys Gly
435 440 445

Phe Val Asp Leu Asn Ser Leu Arg Gln Arg His Asp Ser Phe Arg Ser
450 455 460

Ser Val Gly Thr Tyr Ser Met Asn Ser Ser Arg Gln Thr Arg Asp Thr
465 470 475 480

Glu Lys Asp Glu Phe Trp Lys Val Leu Arg Lys Gln Ser Val Pro Ala
485 490 495

Asn Ile Leu Ser Leu Gln Leu Gln Gln Met Ala Ala Asn Leu Glu Asn
500 505 510

Asp Glu Asp Val Pro Gln Val Thr Gly His Gln Phe Ser Val Leu Gly
515 520 525

Gln Ile His Ile Asp Leu Ser Arg Tyr His Glu Leu Gly Arg Phe Val
530 535 540

```
Glu Val Asp Ser Glu His Lys Glu Met Leu Glu Gly Ser Glu Asn Asp
545                 550                 555                 560

Ala Arg Val Pro Ile Lys Tyr Asp Lys Gln Ser Ala Ile Phe His Leu
                565                 570                 575

Asp Ile Ala Arg Lys Cys Gly Ile Leu Glu Ala Val Leu Thr Ser Ala
            580                 585                 590

His Ile Val Leu Gly Leu Pro His Glu Leu Leu Lys Glu Val Thr Val
        595                 600                 605

Asp Asp Leu Phe Pro Asn Gly Phe Gly Glu Gln Glu Asn Gly Ile Arg
    610                 615                 620

Ala Asp Lys Gly Gln Lys Pro Cys Asp Leu Glu Glu Phe Gly Ser Asp
625                 630                 635                 640

Leu Met Glu Ile Ala Ala Glu Met Gly Asp Lys Gly Ala Met Leu Tyr
                645                 650                 655

Met Ala His Ala Tyr Glu Thr Gly Gln His Leu Gly Pro Asn Arg Arg
            660                 665                 670

Thr Asp Tyr Lys Lys Ser Ile Asp Trp Tyr Gln Arg Val Val Gly Phe
        675                 680                 685

Gln Glu Glu Glu Glu Leu Asp Ser Asp Cys Gly Lys Thr Thr Phe Ser
    690                 695                 700

Ser Phe Ala Pro Leu Thr Arg His Glu Ile Leu Ala Lys Met Ala Glu
705                 710                 715                 720

Met Tyr Lys Glu Gly Gly Tyr Gly Leu Asn Gln Asp Phe Glu Arg Ala
                725                 730                 735

Tyr Gly Leu Phe Asn Glu Ala Ala Glu Ala Ala Met Glu Ala Met Asn
            740                 745                 750

Gly Lys Leu Ala Asn Lys Tyr Tyr Glu Lys Ala Glu Met Cys Gly Glu
        755                 760                 765
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2283 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: C. elegans

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
ATGACGATCG ACACAACAAA TGAGAGCGAC AATAGTCCAA CTAACTCACC AGGATTGGAG     60
GCCTCGGCTC GGACATTCTC GCTCAATGCG TCAAAAATGG TTCGGATAAC CGACGACTAC    120
GCAGATGAAG TGTTCATTGA ACAGAATGAT GTCGTTATCG AGAAGCCTCG TATGGATCCT    180
CTCCACGTTA GAAAACTTAT GGAGACATGG CGCAAGGCTG CTCGCCGAGC AAGAACAAAC    240
TATATAGATC CATGGGATGA GTTCAACATC CACGAGTATC CAGTACAACG AGCTAAACGA    300
TATAGGTATT CTGCAATCAG AAAGCAATGG ACAGAGGATA TAGTCGATGT GAGACTTCAT    360
CCGGACAGTT TTGCACGTGG AGCCATGCGA GAATGCTACC GACTCAAAAA GTGCTCCAAG    420
CACGGAACAA GTCAAGATTG GAGCAGCAAC TATGTCGCAA AAAGATACAT TTGTCAAGTC    480
GATCGTAGAG TTCTTTTCGA TGATGTCAGA CTTCAGATGG ATGCCAAATT ATGGGCTGAA    540
GAATATAATC GGTATAATCC ACCGAAGAAA ATTGATATTG TTCAAATGTG TGTCATTGAG    600
ATGATTGATG TAAAAGGTTC TCCACTCTAT CATTTGGAGC ATTTCATCGA GGGAAAATAT    660
ATAAAATACA ATTCAAACTC AGGATTTGTA TCAAATGCAG CTCGTCTTAC ACCACAAGCA    720
TTTTCTCACT TCACCTTCGA ACGTTCTGGT CATCAAATGA TGGTTGTCGA TATTCAAGGA    780
GTTGGTGATC TTTACACAGA TCCTCAGATT CATACAGTTG TGGGAACTGA TTATGGAGAT    840
GGAAACCTCG GAACTCGTGG AATGGCTCTT TTCTTCCATT CACACAGATG TAACGATATT    900
TGTGAGACAA TGGATCTATC AAATTTCGAA CTTTCGCCAC CTGAAATCGA GGCTACCGAA    960
GTTGCGATGG AAGTAGCTGC AAAGCAGAAA AAGTCATGCA TAGTTCCTCC AACTGTGTTC   1020
GAAGCAAGAA GAAATCGAAT TTCAAGTGAA TGTGTACATG TCGAGCATGG TATTTCGATG   1080
GATCAATTGA GAAAAAGGAA GACGTTGAAT CAATCGTCAA CCGATTTGTC AGCAAAGAGT   1140
CACAACGAAG ACTGTGTATG TCCTGAGTGT ATTCCAGTTG TTGAGCAACT CTGTGAGCCT   1200
```

TGCTCCGAAG ATGAAGAGGA CGAAGAAGAA GACTATCCAA GAAGTGAAAA AAGTGGAAAT 1260

AGTCAGAAAA GTCGACGTAG TAGAATGAGC ATTTCAACGA GATCTTCTGG CGATGAATCA 1320

GCATCTCGTC CTAGAAAATG CGGATTTGTA GATTTAAACT CACTTCGTCA GAGACATGAT 1380

AGCTTCAGAA GTTCTGTTGG GACATATTCT ATGAATAGTT CTAGACAAAC CAGAGACACT 1440

GAAAAGGATG AATTCTGGAA GGTTCTTCGA AAACAATCAG TTCCAGCAAA CATTCTATCA 1500

CTTCAACTTC AACAAATGGC TGCTAACCTG GAAAATGATG AAGACGTACC ACAAGTCACC 1560

GGGCATCAGT TCTCTGTCCT CGGTCAGATT CATATTGATC TCTCACGATA TCATGAGCTC 1620

GGGCGGTTCG TAGAAGTTGA TTCAGAACAT AAGGAAATGC TTGAGGGAAG TGAAAATGAC 1680

GCTCGTGTAC CAATCAAATA CGACAAGCAG TCTGCAATTT TCCATTTGGA TATCGCTCGG 1740

AAGTGTGGAA TCCTTGAGGC TGTGCTAACA TCGGCTCATA TTGTTCTCGG ATTACCACAT 1800

GAATTGTTGA AAGAAGTCAC CGTTGATGAT CTGTTTCCTA ATGGGTTTGG AGAACAGGAA 1860

AATGGAATTC GAGACCTAGA AGAGTTCGGC TCCGATCTGA TGGAAATTGC TGCAGAGATG 1920

GGTGATAAGG GTGCAATGCT GTACATGGCA CACGCTTATG AAACTGGTCA GCATCTCGGA 1980

CCGAATCGAA GAACGGATTA TAAGAAATCG ATTGATTGGT ATCAACGCGT CGTTGGATTC 2040

CAAGAAGAAG AAGAACTTGA CTCTGATTGT GGAAAAACGA CATTCTCCTC ATTTGCTCCA 2100

CTGACTCGTC ACGAGATTCT AGCCAAAATG GCTGAAATGT ACAAAGAGGG AGGTTATGGC 2160

CTGAATCAAG ACTTCGAACG AGCATATGGT CTATTCAATG AAGCTGCTGA AGCAGCAATG 2220

GAAGCAATGA ATGGAAAGCT CGCAAATAAA TACTATGAAA AAGCGGAAAT GTGTGGAGAA 2280

TGA 2283

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 760 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: C. elegans

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Met Thr Ile Asp Thr Thr Asn Glu Ser Asp Asn Ser Pro Thr Asn Ser
1               5                   10                  15

Pro Gly Leu Glu Ala Ser Ala Arg Thr Phe Ser Leu Asn Ala Ser Lys
            20                  25                  30

Met Val Arg Ile Thr Asp Asp Tyr Ala Asp Glu Val Phe Ile Glu Gln
        35                  40                  45

Asn Asp Val Val Ile Glu Lys Pro Arg Met Asp Pro Leu His Val Arg
    50                  55                  60

Lys Leu Met Glu Thr Trp Arg Lys Ala Ala Arg Arg Ala Arg Thr Asn
65                  70                  75                  80

Tyr Ile Asp Pro Trp Asp Glu Phe Asn Ile His Glu Tyr Pro Val Gln
                85                  90                  95

Arg Ala Lys Arg Tyr Arg Tyr Ser Ala Ile Arg Lys Gln Trp Thr Glu
            100                 105                 110

Asp Ile Val Asp Val Arg Leu His Pro Asp Ser Phe Ala Arg Gly Ala
        115                 120                 125

Met Arg Glu Cys Tyr Arg Leu Lys Lys Cys Ser Lys His Gly Thr Ser
    130                 135                 140

Gln Asp Trp Ser Ser Asn Tyr Val Ala Lys Arg Tyr Ile Cys Gln Val
145                 150                 155                 160

Asp Arg Arg Val Leu Phe Asp Asp Val Arg Leu Gln Met Asp Ala Lys
                165                 170                 175

Leu Trp Ala Glu Glu Tyr Asn Arg Tyr Asn Pro Pro Lys Lys Ile Asp
            180                 185                 190

Ile Val Gln Met Cys Val Ile Glu Met Ile Asp Val Lys Gly Ser Pro
        195                 200                 205

Leu Tyr His Leu Glu His Phe Ile Glu Gly Lys Tyr Ile Lys Tyr Asn
    210                 215                 220

Ser Asn Ser Gly Phe Val Ser Asn Ala Ala Arg Leu Thr Pro Gln Ala
225                 230                 235                 240
```

Phe Ser His Phe Thr Phe Glu Arg Ser Gly His Gln Met Met Val Val
245 250 255

Asp Ile Gln Gly Val Gly Asp Leu Tyr Thr Asp Pro Gln Ile His Thr
260 265 270

Val Val Gly Thr Asp Tyr Gly Asp Gly Asn Leu Gly Thr Arg Gly Met
275 280 285

Ala Leu Phe Phe His Ser His Arg Cys Asn Asp Ile Cys Glu Thr Met
290 295 300

Asp Leu Ser Asn Phe Glu Leu Ser Pro Pro Glu Ile Glu Ala Thr Glu
305 310 315 320

Val Ala Met Glu Val Ala Ala Lys Gln Lys Lys Ser Cys Ile Val Pro
325 330 335

Pro Thr Val Phe Glu Ala Arg Arg Asn Arg Ile Ser Ser Glu Cys Val
340 345 350

His Val Glu His Gly Ile Ser Met Asp Gln Leu Arg Lys Arg Lys Thr
355 360 365

Leu Asn Gln Ser Ser Thr Asp Leu Ser Ala Lys Ser His Asn Glu Asp
370 375 380

Cys Val Cys Pro Glu Cys Ile Pro Val Val Glu Gln Leu Cys Glu Pro
385 390 395 400

Cys Ser Glu Asp Glu Glu Asp Glu Glu Glu Asp Tyr Pro Arg Ser Glu
405 410 415

Lys Ser Gly Asn Ser Gln Lys Ser Arg Arg Ser Arg Met Ser Ile Ser
420 425 430

Thr Arg Ser Ser Gly Asp Glu Ser Ala Ser Arg Pro Arg Lys Cys Gly
435 440 445

Phe Val Asp Leu Asn Ser Leu Arg Gln Arg His Asp Ser Phe Arg Ser
450 455 460

Ser Val Gly Thr Tyr Ser Met Asn Ser Ser Arg Gln Thr Arg Asp Thr
465 470 475 480

Glu Lys Asp Glu Phe Trp Lys Val Leu Arg Lys Gln Ser Val Pro Ala
485 490 495

Asn Ile Leu Ser Leu Gln Leu Gln Gln Met Ala Ala Asn Leu Glu Asn
500 505 510

Asp Glu Asp Val Pro Gln Val Thr Gly His Gln Phe Ser Val Leu Gly
515 520 525

Gln Ile His Ile Asp Leu Ser Arg Tyr His Glu Leu Gly Arg Phe Val
530 535 540

Glu Val Asp Ser Glu His Lys Glu Met Leu Glu Gly Ser Glu Asn Asp
545 550 555 560

Ala Arg Val Pro Ile Lys Tyr Asp Lys Gln Ser Ala Ile Phe His Leu
565 570 575

Asp Ile Ala Arg Lys Cys Gly Ile Leu Glu Ala Val Leu Thr Ser Ala
580 585 590

His Ile Val Leu Gly Leu Pro His Glu Leu Leu Lys Glu Val Thr Val
595 600 605

Asp Asp Leu Phe Pro Asn Gly Phe Gly Glu Gln Glu Asn Gly Ile Arg
610 615 620

Asp Leu Glu Glu Phe Gly Ser Asp Leu Met Glu Ile Ala Ala Glu Met
625 630 635 640

Gly Asp Lys Gly Ala Met Leu Tyr Met Ala His Ala Tyr Glu Thr Gly
645 650 655

Gln His Leu Gly Pro Asn Arg Arg Thr Asp Tyr Lys Lys Ser Ile Asp
660 665 670

Trp Tyr Gln Arg Val Val Gly Phe Gln Glu Glu Glu Glu Leu Asp Ser
675 680 685

Asp Cys Gly Lys Thr Thr Phe Ser Ser Phe Ala Pro Leu Thr Arg His
690 695 700

Glu Ile Leu Ala Lys Met Ala Glu Met Tyr Lys Glu Gly Gly Tyr Gly
705 710 715 720

Leu Asn Gln Asp Phe Glu Arg Ala Tyr Gly Leu Phe Asn Glu Ala Ala
725 730 735

Glu Ala Ala Met Glu Ala Met Asn Gly Lys Leu Ala Asn Lys Tyr Tyr
740 745 750

Glu Lys Ala Glu Met Cys Gly Glu
755 760

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 628 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Dictyostelium discoideum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
GTATTGTATG TGTTTCAATT GAGAAGACTC CATTTGCAAA GGGTAGTTGT AGAACAGCAC      60

ATAAATTAAA GGATTGGAGT CAACCAGATC AAGGATTAGT TGGTAAATTT TCAACCAATA     120

AAAAGACGAC AAGAGATTCA TACTTTACAG ATGTATTGAT GCAAACATTT TGTGCTAAAT     180

GGGCAGAGAA ATTCAATGAA GCGAAACCAC CAAAACCAAT TACATTCTTA CCATCATACG     240

TTTACGAATT GATTGATCAT CCACCACCTT ATCCAGTTTG TGGTGGTGAA CCATTCATTG     300

AGGGAGATTA CAAGAAACAT AACAACAACA GTGGTTACGT TAGTAGTGAT GCTAGAAATA     360

CACCACAATC ATTCTCTCAT TTCTCATACG AACTCTCCAA TCATGAATTG TTGATCGTTG     420

ATATCCAAGG TGTCAACGAT TTCTACACTG ATCCTCAAAT TCATACGAAA TCAGGCGAGG     480

GCTTTGGCGA GGGTAATTTG GGCGAGACGG GTTTCCACAA ATTCCTTCAA ACACACAAGT     540

GTAATCCAGT TTGTGACTTT TTAAAGTTGA AACCAATCAA TCAATCAAAG AAAGCCCTCC     600

TAAGAGGTAC ATTACCCGTC GTACAATT                                        628
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 209 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Dictyostelium discoideum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Ile Val Cys Val Ser Ile Glu Lys Thr Pro Phe Ala Lys Gly Ser Cys
1               5                   10                  15

Arg Thr Ala His Lys Leu Lys Asp Trp Ser Gln Pro Asp Gln Gly Leu
            20                  25                  30

Val Gly Lys Phe Ser Thr Asn Lys Lys Thr Thr Arg Asp Ser Tyr Phe
        35                  40                  45

Thr Asp Val Leu Met Gln Thr Phe Cys Ala Lys Trp Ala Glu Lys Phe
    50                  55                  60

Asn Glu Ala Lys Pro Pro Lys Pro Ile Thr Phe Leu Pro Ser Tyr Val
65                  70                  75                  80

Tyr Glu Leu Ile Asp His Pro Pro Pro Tyr Pro Val Cys Gly Gly Glu
                85                  90                  95

Pro Phe Ile Glu Gly Asp Tyr Lys Lys His Asn Asn Asn Ser Gly Tyr
            100                 105                 110

Val Ser Ser Asp Ala Arg Asn Thr Pro Gln Ser Phe Ser His Phe Ser
        115                 120                 125

Tyr Glu Leu Ser Asn His Glu Leu Leu Ile Val Asp Ile Gln Gly Val
    130                 135                 140

Asn Asp Phe Tyr Thr Asp Pro Gln Ile His Thr Lys Ser Gly Glu Gly
145                 150                 155                 160

Phe Gly Glu Gly Asn Leu Gly Glu Thr Gly Phe His Lys Phe Leu Gln
                165                 170                 175

Thr His Lys Cys Asn Pro Val Cys Asp Phe Leu Lys Leu Lys Pro Ile
            180                 185                 190

Asn Gln Ser Lys Lys Ala Leu Leu Arg Gly Thr Leu Pro Val Val Gln
        195                 200                 205

Leu
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 238 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Gly Glu Trp Leu Asp Asp Glu Val Leu Ile Lys Met Ala Ser Gln Pro
1               5                   10                  15

Phe Gly Arg Gly Ala Met Arg Glu Cys Phe Arg Thr Lys Lys Leu Ser
            20                  25                  30

Asn Phe Leu His Ala Gln Gln Trp Lys Gly Ala Ser Asn Tyr Val Ala
        35                  40                  45

Lys Arg Tyr Ile Glu Pro Val Asp Arg Asp Val Tyr Phe Glu Asp Val
    50                  55                  60

Arg Leu Gln Met Glu Ala Lys Leu Trp Gly Glu Glu Tyr Asn Arg His
65                  70                  75                  80

Lys Pro Pro Lys Gln Val Asp Ile Met Gln Met Cys Ile Ile Glu Leu
                85                  90                  95

Lys Asp Arg Pro Gly Lys Pro Leu Phe His Leu Glu His Tyr Ile Glu
            100                 105                 110

Gly Lys Tyr Ile Lys Tyr Asn Ser Asn Ser Gly Phe Val Arg Asp Asp
        115                 120                 125

Asn Ile Arg Leu Thr Pro Gln Ala Phe Ser His Phe Thr Phe Glu Arg
    130                 135                 140

Ser Gly His Gln Leu Ile Val Val Asp Ile Gln Gly Val Gly Asp Leu
145                 150                 155                 160

Tyr Thr Asp Pro Gln Ile His Thr Glu Thr Gly Thr Asp Phe Gly Asp
                165                 170                 175

Gly Asn Leu Gly Val Arg Gly Met Ala Leu Phe Phe Tyr Ser His Ala
            180                 185                 190
```

```
Cys Asn Arg Ile Cys Glu Ser Met Gly Leu Ala Pro Phe Asp Leu Ser
        195                     200                     205

Pro Arg Glu Arg Asp Ala Val Asn Gln Asn Thr Lys Leu Leu Gln Ser
    210                     215                     220

Ala Lys Thr Ile Leu Arg Gly Thr Glu Glu Lys Cys Gly Ser
225                     230                     235
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 258 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: D. discoideum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Asn Lys Trp Ile Arg Leu Ser Met Lys Leu Lys Val Glu Arg Lys Pro
1               5                   10                  15

Phe Ala Glu Gly Ala Leu Arg Glu Ala Tyr His Thr Val Ser Leu Gly
            20                  25                  30

Val Gly Thr Asp Glu Asn Tyr Pro Leu Gly Thr Thr Thr Lys Leu Phe
        35                  40                  45

Pro Pro Ile Glu Met Ile Ser Pro Ile Ser Lys Asn Asn Glu Ala Met
    50                  55                  60

Thr Gln Leu Lys Asn Gly Thr Lys Phe Val Leu Lys Leu Tyr Lys Lys
65                  70                  75                  80

Glu Ala Glu Gln Gln Ala Ser Arg Glu Leu Tyr Phe Glu Asp Val Lys
                85                  90                  95

Met Gln Met Val Cys Arg Asp Trp Gly Asn Lys Phe Asn Gln Lys Lys
            100                 105                 110

Pro Pro Lys Lys Ile Glu Phe Leu Met Ser Trp Val Val Glu Leu Ile
        115                 120                 125
```

Asp Arg Ser Pro Ser Ser Asn Gly Gln Pro Ile Leu Cys Ser Ile Glu
130 135 140

Pro Leu Leu Val Gly Glu Phe Lys Lys Asn Asn Ser Asn Tyr Gly Ala
145 150 155 160

Val Leu Thr Asn Arg Ser Thr Pro Gln Ala Phe Ser His Phe Thr Tyr
165 170 175

Glu Leu Ser Asn Lys Gln Met Ile Val Val Asp Ile Gln Gly Val Asp
180 185 190

Asp Leu Tyr Thr Asp Pro Gln Ile His Thr Pro Asp Gly Lys Gly Phe
195 200 205

Gly Leu Gly Asn Leu Gly Lys Ala Gly Ile Asn Lys Phe Ile Thr Thr
210 215 220

His Lys Cys Asn Ala Val Cys Ala Leu Leu Asp Leu Asp Val Lys Leu
225 230 235 240

Gly Gly Val Leu Ser Gly Asn Asn Lys Lys Gln Leu Gln Gln Gly Thr
245 250 255

Met Val

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 212 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: D. discoideum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ala Gln Trp Thr Cys Thr Ala Thr Leu Val Lys Val Glu Pro Val Pro
1 5 10 15

Phe Ala Glu Gly Ala Phe Arg Lys Ala Tyr His Thr Leu Asp Leu Ser
20 25 30

```
Lys Ser Gly Ala Ser Gly Arg Tyr Val Ser Lys Ile Gly Lys Lys Pro
        35                  40                  45

Thr Pro Arg Pro Ser Tyr Phe Glu Asp Val Lys Met Gln Met Ile Ala
    50                  55                  60

Lys Lys Trp Ala Asp Lys Tyr Asn Ser Phe Lys Pro Pro Lys Lys Ile
65                  70                  75                  80

Glu Phe Leu Gln Ser Cys Val Leu Glu Phe Val Asp Arg Thr Ser Ser
                85                  90                  95

Asp Leu Ile Cys Gly Ala Glu Pro Tyr Val Glu Gly Gln Tyr Arg Lys
            100                 105                 110

Tyr Asn Asn Asn Ser Gly Phe Val Ser Asn Asp Glu Arg Asn Thr Pro
        115                 120                 125

Gln Ser Phe Ser His Phe Thr Tyr Glu His Ser Asn His Gln Leu Leu
    130                 135                 140

Ile Ile Asp Ile Gln Gly Val Gly Asp His Tyr Thr Asp Pro Gln Ile
145                 150                 155                 160

His Thr Tyr Asp Gly Val Gly Phe Gly Ile Gly Asn Leu Gly Gln Lys
                165                 170                 175

Gly Phe Glu Lys Phe Leu Asp Thr His Lys Cys Asn Ala Ile Cys Gln
            180                 185                 190

Tyr Leu Asn Leu Gln Ser Ile Asn Pro Lys Ser Glu Lys Ser Asp Cys
        195                 200                 205

Gly Thr Val Pro
    210
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 233 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
- (A) ORGANISM: C. elegans

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Lys Gln Trp Thr Glu Asp Ile Val Asp Val Arg Leu His Pro Asp Ser
1               5                   10                  15

Phe Ala Arg Gly Ala Met Arg Glu Cys Tyr Arg Leu Lys Lys Cys Ser
            20                  25                  30

Lys His Gly Thr Ser Gln Asp Trp Ser Ser Asn Tyr Val Ala Lys Arg
        35                  40                  45

Tyr Ile Cys Gln Val Asp Arg Arg Val Leu Phe Asp Asp Val Arg Leu
    50                  55                  60

Gln Met Asp Ala Lys Leu Trp Ala Glu Glu Tyr Asn Arg Tyr Asn Pro
65                  70                  75                  80

Pro Lys Lys Ile Asp Ile Val Gln Met Cys Val Ile Glu Met Ile Asp
                85                  90                  95

Val Lys Gly Ser Pro Leu Tyr His Leu Glu His Phe Ile Glu Gly Lys
            100                 105                 110

Tyr Ile Lys Tyr Asn Ser Asn Ser Gly Phe Val Ser Asn Ala Ala Arg
        115                 120                 125

Leu Thr Pro Gln Ala Phe Ser His Phe Thr Phe Glu Arg Ser Gly His
    130                 135                 140

Gln Met Met Val Val Asp Ile Gln Gly Val Gly Asp Leu Tyr Thr Asp
145                 150                 155                 160

Pro Gln Ile His Thr Val Val Gly Thr Asp Tyr Gly Asp Gly Asn Leu
                165                 170                 175

Gly Thr Arg Gly Met Ala Leu Phe Phe His Ser His Arg Cys Asn Asp
            180                 185                 190

Ile Cys Glu Thr Met Asp Leu Ser Asn Phe Glu Leu Ser Pro Pro Glu
        195                 200                 205

Ile Glu Ala Thr Glu Val Ala Met Glu Val Ala Ala Lys Gln Lys Lys
    210                 215                 220

Ser Cys Ile Val Pro Pro Thr Val Phe
225                 230
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotide Primer D"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGATTTGGAC TGGACAAGAA CCCCC 25

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Arg Lys Lys Phe Gly Glu Ser Glu Lys Thr Lys Thr Lys Glu Phe Leu
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Leu Thr Pro Gln Ala Phe Ser His Phe Thr Phe Glu Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Leu Ala Asn Xaa Tyr Tyr Glu Lys Ala Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotides"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CANGCNTTNN NNCANTTNAC NTTNGANNG 29

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotides"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TCNGCNTTNT CNTANTANTT NTTNGC 26

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligonucleotides"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TACAATCAGC TGATGACCAG AACGCTC 27

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(57) Abstract

A new superfamily of protein kinases has been discovered that centers around eukaryotic elongation factor-2 kinase (eEF-2 kinase). The proetin kinases of this new superfamily have the following characteristics: 1) sequence similarity to eEF-2 kinase; 2) no sequence similarity to the protein kinases of either the serine/threonine/tyrosine kinase or histidine kinase superfamily; and, 3) specifically phosphorylates α-helical regions of proteins as opposed to β-turns, as seen in all other protein kinases. Assays have been developed utilizing eEF-2 kinase and a phosphorylation target consisting of a novel α-helical 16-amino acid peptide sequence to facilitate high-throughput screening for compounds that can specifically inhibit this protein kinase that has been implicated tumor growth and other hyperproliferative disorders. Additionally, the disclosed invention includes assessing eEF-2 kinase levels for diagnostic purposes, and therapeutic formulations to inhibit eEG-2 kinase activity.

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INTERNATIONAL SEARCH REPORT

International Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12P21/02 C07K14/00 C07K16/00 C12N15/11 C12N1/11
C12N1/15 C12N1/19 C12N1/21 C12N15/63

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12P C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	W. HAIT ET AL.: "Elongation vfactor-2 kinase: immunological evidence for the existence of tissue specific forms" FEBS LETTERS, vol. 397, 1996, pages 55-60, XP002097685 *see the whole article* ---	1-51
X	G.P. COTE ET AL.: "Mapping of the novel protein kinase catalytic domain of Dictyostelium myosin II heavy chain kinase A" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 11, 1997, pages 6846-6849, XP002097686 *see the whole article* ---	1-51

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[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
24 March 1999	20/04/1999
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Marie, A

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	S. RAVID ET AL.: "Membrane bound Dictyostelium myosin heavy chain kinase: a developmentally regulated substrate specific member of the protein kinase C family" PNAS, vol. 89, 1992, pages 5877-5881, XP002097687 *see the whole article* ---	1-51
X	L.M. FUTEY ET AL.: "Structural analysis of myosin heavy chain kinase A" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 2, 1995, pages 523-529, XP002097688 *see the whole article* ---	1-51
X	C.E. CLANCY ET AL.: "Identification of a protein kinase from Dictyostelium with homology to the novel catalytic domain of myosin heavy chain kinase A" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 18, 1997, pages 11812-11815, XP002097689 *see the whole article* ---	1-51
X	Proceedings of the 88th annual meeting of the american association for cancer research.1997, 38, abstract 2806 XP002097696 *see the whole abstract* ---	1-51
X	Proceedings of the 88th annual meeting of the american assoication for cancer research, 1997, 38, abstract 3762 XP002097697 *see the whole abstract* ---	1-51
X	Proceedings of the 88th annual meeting of the american association foir cancer research, 1997, 38, abstract 3761 XP002097698 *see the whole abstract* ---	1-51
X	N.T. REDPATH ET AL.: "Cloning and expression of cDNA encoding protein synthesis leongation factor-2 kinase" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 29, 1996, pages 17547-17554, XP002097690 *see the whole article* ---	1-51
	-/--	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	A.G. RYAZANOV ET AL.: "Identification of a new class of protein kinases represented by eukaryotic elongation factor-2 kinase" PNAS, vol. 94, 1997, pages 4884-4889, XP002097691 *see the whole article* ---	1-51
X	K. MITSUI ET AL.: "Purification and characterization of calmodulin dependent protein kinase III fron rabbit reticulocytes and rat pancreas" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 268, no. 18, 1993, pages 13422-13433, XP002097692 *see the whole article* ---	1-51
X	N.T. REDPATH ET AL.: "Purification and phosphorylation of elongation factor-2 kinase from rabbit reticulocytes" EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 212, 1993, pages 511-520, XP002097693 *see the whole article* ---	1-51
X	A. GAJKO ET AL.: "Purification and characterization of the protein kinase eEF-2 isolated from rat liver cells" ACTA BIOCHIMICA POLONICA, vol. 41, no. 4, 1994, pages 421-427, XP002097694 *see the whole article* ---	1-51
X	A.C. NAIRN ET AL.: "Calcium/calmodulin dependent protein kinases" CANCER BIOLOGY, vol. 5, 1994, pages 295-303, XP002097695 *see the whole article* -----	1-51

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